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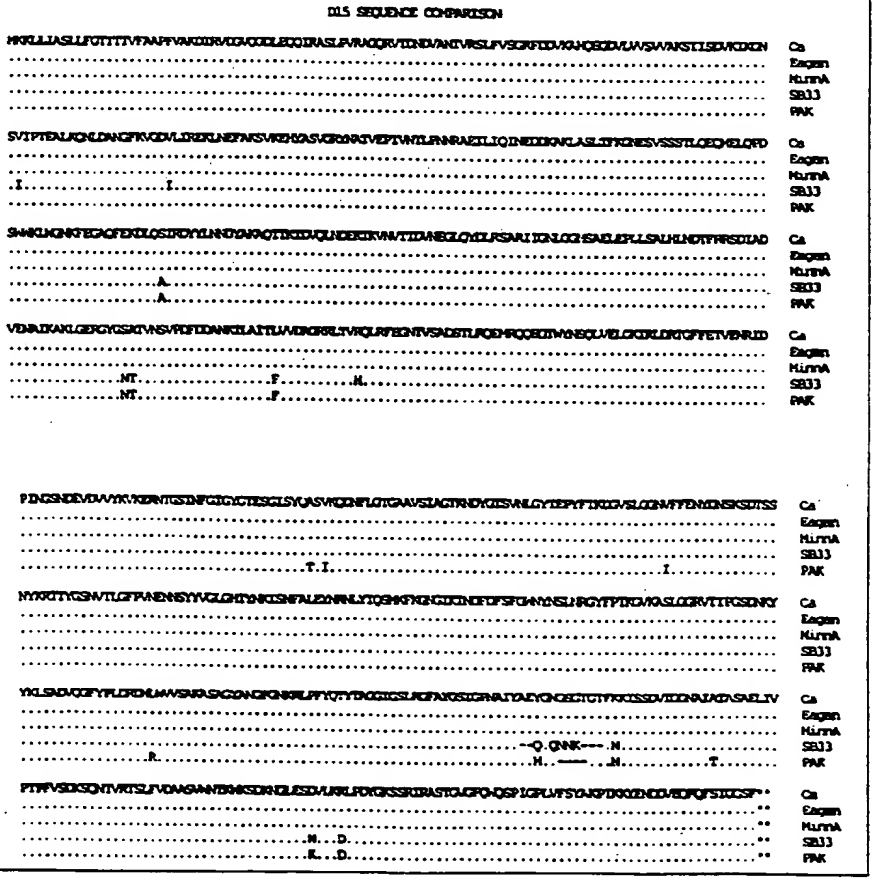
(54) Title: HAEMOPHILUS OUTER MEMBRANE PROTEIN

(57) Abstract

Purified and isolated nucleic acid from specific strains of *Haemophilus influenzae* is provided which encodes at least a portion of the D15 outer membrane protein of *Haemophilus*. The nucleic acid is used to produce peptides, polypeptides and proteins free of contaminant associated with *Haemophilus* for purposes of diagnosis and medical treatment. Furthermore, the nucleic acid may be used in the diagnosis of *Haemophilus* infection. Antisera obtained following immunization with the nucleic acid D15 outer membrane protein or peptides also may be used for the purpose of diagnosis and medical treatment.



TITLE OF INVENTION

HAEMOPHILUS OUTER MEMBRANE PROTEIN

FIELD OF INVENTION

The present invention is related to the field of molecular genetics and is particularly concerned with the cloning of an outer membrane protein D15 of Haemophilus.

BACKGROUND OF THE INVENTION

Haemophilus influenzae type b (Hib) is a major cause of bacterial meningitis in children under the age of five years. Protective antibodies to the disease are induced by the capsular polysaccharide of the organism and a vaccine was developed that utilises the purified polyribosyl ribitol phosphate (PRP) as the antigen. This vaccine provides 90% protection in adults and in children over 24 months of age, but was ineffective in children under 24 months Zangwill et al 1993 (The references are identified in a list of reference at the end of this disclosure). Like other polysaccharide antigens, PRP does not induce the proliferation of T-helper cells, and re-immunisation fails to elicit either a booster response or an increase in memory cells. Conjugation of the PRP polysaccharide with protein carriers confers T-cell dependent characteristics to the vaccine and substantially enhances the immunologic response to the PRP antigen. Currently, there are four PRP-carrier conjugate vaccines available. These are vaccines based upon H. influenzae type b capsular polysaccharide conjugated to diphtheria toxoid, tetanus toxoid, or Neisseria meningitidis outer membrane protein (reviewed in Zangwill et al 1993).

However, the current Haemophilus conjugate vaccines only protect against meningitis caused by Haemophilus influenzae type b. They do not protect against other invasive typeable strains (types a and c) and, more importantly, against non-typeable (NTHi) strains which are a common cause of postpartum and neonatal sepsis,

otitis media, epiglottitis, pneumonia, and tracheobronchitis, are required.

SUMMARY OF THE INVENTION

The present invention is directed towards the provision of purified and isolated nucleic acid molecules comprising at least a portion coding for a D15 outer membrane protein of a species of Haemophilus. The nucleic acid molecules comprising at least a portion coding for D15 outer membrane protein are useful for the specific detection of strains of Haemophilus, and for diagnosis of infection by Haemophilus. The purified and isolated nucleic acid molecules, such as DNA comprising at least a portion coding for D15 outer membrane protein, are also useful for expression of the D15 gene by recombinant DNA means for providing, in an economical manner, purified and isolated D15 outer membrane protein.

The D15 outer membrane protein or fragments thereof or analogs thereof are useful immunogenic compositions for the preparation of vaccines against diseases caused by Haemophilus, the diagnosis of infection by Haemophilus and as tools for the generation of immunological reagents. Mono- or polyclonal antisera (antibodies) raised against the D15 outer membrane protein produced in accordance with aspects of the present invention are useful for the diagnosis of infection by Haemophilus, specific detection of Haemophilus (in, for example, in vitro and in vivo assays) and for the treatment of diseases caused by infection by Haemophilus.

Peptides corresponding to portions of the D15 outer membrane protein or analogs thereof are useful immunogenic compositions for the preparation of vaccines against disease caused by Haemophilus, the diagnosis of infection by Haemophilus and as tools for the generation of immunological reagents. Mono- or polyclonal antisera raised against these peptides, produced in accordance with aspects of the present invention, are useful for the

comprises at least an 18 bp fragment selected from the DNA molecules as recited above is inserted. The recombinant plasmid may be plasmid DS-712-2-1 having ATCC accession number 75604, deposited November 4, 1993 and plasmid JB-1042-5-1 having ATCC accession number 75006, deposited November 4, 1993.

The plasmids may be adapted for expression of the encoded D15 outer membrane protein in a host cell, which may be a heterologous or homologous host, by incorporation into a recombinant vector, provided in accordance with a further aspect of the invention. The recombinant vector may comprise at least a DNA segment comprising at least an 18 bp fragment selected from the DNA molecules as recited above and expression means operatively coupled to the DNA segment for expression of the gene product encoded thereby in the host cell. The plasmid for expression of the encoded D15 outer membrane protein may be plasmid DS-880-1-2 having ATCC accession number 75605, deposited November 4, 1993 being adapted for expression at the D15 outer membrane protein in E. coli. The selected DNA segment may encode a polypeptide of at least 6 residues and, in particular, may be selected from those segments encoding a polypeptide of Table 2 (below). The DNA segment may further comprise a nucleic acid sequence encoding a leader sequence for export of the gene product from the host. The host for expression may be selected from, for example, Escherichia coli, Bacillus, Haemophilus, fungi, yeast or the baculovirus expression system may be used.

Additional aspects of the invention include the protein encoded by the DNA molecule comprising at least a portion coding for the D15 outer membrane protein, fragment or a functional analog of such protein, the use of the protein or analog in vaccination and diagnosis, and the generation of immunological reagents. The invention also includes antisera (antibodies) raised

of the immunogenic composition or the nucleic acid molecule as recited above to provide protective immunity against Haemophilus infection.

The present invention further includes a chimeric molecule comprising a D15 protein or peptide corresponding thereto as provided herein linked to another polypeptide or protein or a polysaccharide. The linked polypeptide or protein may comprise a surface protein or peptide corresponding thereto from a pathogenic bacteria, which may be the P1, P2 or P6 outer membrane protein of H. influenzae. The linked polysaccharide preferably comprise a PRP molecule from H. influenzae.

BRIEF DESCRIPTION OF THE FIGURES

The present invention will be further understood from the following description with reference to the drawings, in which:

Figure 1A shows the nucleotide sequence of the D15 gene from H. influenzae type b Ca strain (SEQ ID NO: 1) and its deduced amino acid sequence (SEQ ID NO: 2);

Figure 1B shows the nucleotide sequence of the D15 gene from H. influenzae type b Eagan strain (SEQ ID NO. 3) and its deduced amino acid sequence (SEQ ID NO: 4);

Figure 1C shows the nucleotide sequence of the D15 gene from H. influenzae type b MinnA strain (SEQ ID NO. 5) and its deduced amino acid sequence (SEQ ID NO: 6);

Figure 1D shows the nucleotide sequence of the D15 gene from H. influenzae non-typeable SB33 (SEQ ID NO. 7) and its deduced amino acid sequence (SEQ ID NO: 8);

Figure 1E shows the nucleotide sequence of the D15 gene from H. influenzae non-typeable PAK 12085 (SEQ ID NO. 9) and its deduced amino acid sequence (SEQ ID NO: 10);

Figure 1F shows an alignment of the nucleotide sequences of the D15 genes (SEQ ID NOS: 1, 3, 5, 7 and 9)

97kDa); 2, GST standard; 3, GST-(D15 fragment) fusion protein; 4, fusion protein cleaved by thrombin; 5, N-terminal rD15 fragment; 6, GST; 7, low molecular weight markers;

Figure 10 shows guinea pig IgG antibody response to N-terminal rD15 fragment. The arrows indicate the immunization schedule. Bleeds were taken at 2, 4, 6 and 8 weeks. The bars represent the standard deviation; and

Figure 11 shows the hydrophilicity plot of D15 established by using a window average across 7 residues according to Hope, 1986.

GENERAL DESCRIPTION OF THE INVENTION

Any Haemophilus strains that have D15 genes may be conveniently used to provide the purified and isolated nucleic acid molecules (which may be in the form of DNA molecules), comprising at least a portion coding for a D15 outer membrane protein as typified by embodiments of the present invention. Such strains are generally available from clinical sources and from bacterial culture collections, such as the American Type Culture Collection. H. influenzae strains may include types a, b and c strains, non-typeable strains and other bacteria that produce a D15 protein, fragment or analog thereof. Appropriate strains of Haemophilus include:-

H. influenzae type b strain Ca;
H. influenzae type b strain MinnA;
H. influenzae type b strain Egan;
H. influenzae non-typeable b strain SB33; or
H. influenzae non-typeable b strain PAK 12085.

In this application, the term D15 outer membrane protein is used to define a family of D15 proteins which includes those having naturally occurring variations in their amino acid sequences as found in various strains of, for example, Haemophilus. The purified and isolated DNA molecules comprising at least a portion coding for D15 outer membrane protein of the present invention also

described herein are advantageous as diagnostic reagents, antigens for the production of Haemophilus-specific antisera, for vaccination against the diseases caused by species of Haemophilus and for detecting infection by Haemophilus.

Reference will now be made in detail to the presently preferred embodiments of the invention, which together with the following Examples, serve to explain the principle of the invention. For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the following sections:

(i) The DNA sequences coding for the outer membrane protein D15 from H. influenzae type b Ca strain.

A clone producing the outer membrane protein designated D15 of H. influenzae type b (Hib) was isolated by screening a genomic library with H. influenzae type b OMP-specific polyclonal antibodies as previously described by Berns and Thomas 1965; Thomas and Rossi 1986. The DNA fragment encoding the D15 protein was isolated, subcloned into pUC19 to produce pUC19/D15 (Figure 2) and used to transform E. coli HB101 as described in Example 1. Plasmid DNA was prepared from two individual colonies of E. coli HB101 containing the pUC19/D15 plasmid. Sequencing was performed on an ABI DNA sequencer model 370A using dye-terminator chemistry and oligonucleotide primers which had been synthesized on an ABI DNA synthesizer model 380B, and purified by chromatography. Nucleotide sequence analysis of the D15 gene revealed that it contains a putative promoter and an open reading frame encoding 789 amino acids (Figure 1A).

The first 19 amino acid residues of the translated open reading frame form a typical leader sequence as found in other H. influenzae type b outer membrane proteins, such as P1 and P2. The N-terminal sequence of immuno-affinity purified native D15 antigen was

heterologous proteins in E. coli. The T7 expression system is described in U.S. Patent 4,952,496. Clones were, therefore, constructed which utilize the T7 system to express a mature D15 protein that contains an additional methionine residue at the amino terminus. The D15 signal sequence was removed during this construction process. A full length recombinant D15 (termed rD15) was expressed in inclusion bodies which allow the D15 protein to be readily purified. The D15 genes from H. influenzae type b strain Ca and H. influenzae non-typeable SB33 strain have been expressed at high levels in E. coli using the T7 system to permit production of large quantities of rD15 protein. The construction of clone DS-880-1-2 which expresses the SB33 D15 gene is described herein (see Figure 4 and Example 5). The rD15 protein was immunologically similar to its native counterpart isolated from H. influenzae typeable and non-typeable strains (see below). Thus, rD15 may be used as a cross-reactive antigen in a diagnostic kit to detect many, if not all, strains of H. influenzae and other bacteria that produce a D15 outer membrane protein or analog thereof. Alternatively, rD15 can be used as an antigen to specifically detect the presence of H. influenzae in a sample.

A truncated D15 fragment was expressed in E. coli as a fusion protein with glutathione S-transferase (GST), as described in Example 6. The construction was designed to express the N-terminal fragment of the D15 protein. The fusion protein was expressed at high levels from a pGEX-2T construction and the N-terminal fragment was cleaved from the GST carrier protein by treatment with thrombin. This procedure generated a molecule termed the N-terminal rD15 fragment which encompasses amino acids 63-223 of the D15 protein. This N-terminal rD15 fragment was highly immunogenic and elicited protective antibodies against challenge with live H. influenzae.

urea (see Example 8). After dialysis against PBS to remove urea, more than 80% of the D15 protein remained soluble. This soluble rD15 antigen was used for the immunogenicity studies described below. From shake-flask experiments, it was estimated that about 10 mg of soluble rD15 protein was obtained from 1 L of E. coli bacterial culture. It is clear that growing the recombinant E. coli strains under optimised fermentation conditions significantly increase the level of rD15 production.

(vi) Immunogenicity of the full-length recombinant D15 protien (rD15).

The immunogenicity of the full-length rD15 protein was studied in guinea pigs and mice. Using the immunization protocols described in Figure 7, a 15 μg dose of rD15 induced high IgG titers in guinea pigs when administered in the presence of either Freund's adjuvant or $AlPO_4$. In the mouse dose-response study, the protein appeared to be immunogenic at a dose as low as 5 μg in either Freund's adjuvant (Figure 8A) or $AlPO_4$ (Figure 8B).

The protective ability of rD15 against H. influenzae type b infection was examined in the infant rat model of bacteremia essentially as described by Loeb (1987). Thus, infant rats passively immunized with guinea pig anti-rD15 antisera were significantly less bacteremic than controls injected with pre-bleed sera, which is consistent with the previous report by Thomas et al. (1990).

(vii) Purification and characterization of the N-terminal rD15 fragment.

The truncated rD15 fragment corresponding to the N-terminus of the D15 protein (residues 22 to 223) as described in Example 6, was expressed in E. coli as a soluble protein fused to GST. The fusion protein (46 kDa) was readily extracted using phosphate buffered saline (PBS). Purification of the GST-D15 fragment fusion

the immunization protocols described in Figure 10, a 10 μg dose of N-terminal rD15 fragment induced a good booster response in guinea pigs with almost all the adjuvants tested. The highest anti-D15 IgG titer was observed in the group of guinea pigs immunized with N-terminal rD15 fragment in Freund's adjuvant. The second best adjuvant was Titermax (CytRx Inc.). The other two adjuvants, TPAD4 (tripalmityl-Cys-Ser-Glu_4) and $AlPO_4$ were equally potent.

(ix) Protective ability of the N-terminal rD15 fragment against H. influenzae type b challenge.

An in vivo challenge model for a assessing the protective abilities of antigen against diseases caused by Haemophilus is the infant rat model of bacteremia as described by Loeb 1987. The protective ability of the N-terminal rD15 fragment against H. influenzae type b challenge was examined in this rat model. As illustrated in Table 1, infant rats passively immunized with rabbit anti-N-terminal rD15 fragment antisera showed significantly lower bacteremia compared to those injected with pre-bleed sera.

Since passively transferred antisera against the N-terminal rD15 fragment were found to be protective in the infant rat model of bacteremia, it was of interest to identify the protective epitope(s) of this N-terminal rD15 fragment. The first nine overlapping peptides of the D15 protein as listed in Table 2 were chemically synthesized based upon the amino acid sequence derived from the sequence of the D15 gene from H. influenzae type b Ca (Figure 1). These synthetic peptides were assessed for their reactivities with either rabbit or guinea pig antisera raised against purified N-terminal rD15 fragment by ELISAs. As shown in Table 3, both guinea pig and rabbit antisera reacted with a cluster of D15 peptides, including peptides D15-P4 to D15-P8 encompassing residues 93 to 209 of the D15 primary sequence.

was completely blocked by the addition of this mixture of five peptides (Table 5, group #2, 106%, $p = 0.53 \times 10^{-8}$). These results strongly indicate that a cocktail of D15 synthetic peptides may be used as immunogens to induce protective antibodies against H. influenzae.

(x) Epitope prediction and peptide synthesis.

To map the immunodominant T-cell or B-cell epitopes of D15, overlapping synthetic peptides covering the entire D15 protein sequence (Table 2 - SEQ ID NO: 14 to 49) were synthesized using the t-Boc solid-phase peptide synthesis as described in Example 15. The peptides were chosen based on their high index of hydrophillic β-turns estimated by secondary structure prediction analysis (Figure 11). Such peptides are likely to be surface-exposed and antigenic. Peptides more than 25 residues in length were selected to better mimic native epitopes.

(xi) Identification and characterization of immunodominant epitopes of D15 using synthetic peptides.

To map the linear B-cell epitopes of D15, overlapping synthetic peptides representing the entire sequence of D15 were individually coated onto ELISA plates and probed with several anti-rD15 antisera as described in Example 19. The results are summarized in Table 6. Mouse antisera raised against rD15 reacted with all D15 peptides, but the major epitopes were located within peptides D15-P8 (residues 180-209 - SEQ ID NO: 21), D15-P10 (residues 219-249 - SEQ ID NO: 23), D15-P11 (residues 241-270 - SEQ ID NO: 24), and D15-P26 (residues 554-582 - SEQ ID NO: 39), respectively. Rabbit anti-D15 antisera recognized only peptides D15-P4 (residues 93-122 - SEQ ID NO: 17), D15-P14 (residues 304-333 - SEQ ID NO: 27) and D15-P36 (residues 769-798 - SEQ ID NO: 49). Guinea pig antisera raised against rD15 reacted with peptides D15-P2 (residues 45-72 - SEQ ID NO: 15), D15-P4 (residues 93-122 - SEQ ID NO: 17), D15-P6 (residues 135-164 - SEQ ID NO: 19), D15-P8 (residues 180-209 - SEQ ID

cells in the immune system is to provide helper activity for eliciting high levels of antigen-specific antibodies following immunization. Antigens containing Th1 epitope(s) stimulate antigen-specific T-cells to produce high levels of IL-2 and IFN-γ, whereas Th2 epitope(s) induce high levels of IL-4 expression. Th0 epitope(s) stimulate the synthesis of IFN-γ and IL-4.

Little is known about the cellular immune response to outer membrane proteins of H. influenzae and its role in the protection against H. influenzae infection and diseases. To this end, the inventors performed studies of the cellular response elicited in mice following rD15 immunization. D15-specific T-cell epitopes were determined using D15 peptides and T-cell lines obtained from five BALB/c mice immunized with rD15 (see Example 23). The lymphocyte proliferative responses of the D15-specific T-cell lines to overlapping D15 peptides were determined in conventional cytokine assays as described in Example 24. The results summarized in Table 7, revealed that stimulation only with certain synthetic peptides elicited proliferative responses and the release of specific cytokines. Synthetic peptides corresponding to residues 114-143 (D15-P5 - SEQ ID NO: 18), 282-312 (D15-P13 - SEQ ID NO: 26) and 577-602 (D15-P27 - SEQ ID NO: 40), and 219-249 (D15-P10 - SEQ ID NO: 23), 262-291 (D15-P12 - SEQ ID NO: 25), 390-416 (D15-P18 - SEQ ID NO: 31), 410-435 (D15-P19 - SEQ ID NO: 32) 554-582 (D15-P26 - SEQ ID NO: 39), 596-625 (D15-P28 - SEQ ID NO: 41), 725-750 (D15-P34 - SEQ ID NO: 47) and 745-771 (D15-P35 - SEQ ID NO: 48) were shown to be highly stimulatory for rD15-specific BALB/c Th0 cells and Th1 cells, respectively. Therefore, these immunodominant T-cell epitopes can be used as autologous carriers for PRP, and/or OMP B-cell epitopes to enhance their immunogenicity. The Th1 cell epitopes identified above may be useful in the H.

linked to polysaccharides including PRP as synthetic glycopeptide or lipoglycopeptide conjugates to produce alternate vaccines. These vaccines can be used to immunize against diseases caused by H. influenzae when administered to mammals, for example, by the intramuscular or parenteral route, or when delivered using microparticles, capsules, liposomes and targeting molecules, such as toxins or fragments thereof, and antibodies, to cells of the immune system or mucosal surfaces.

(xiv) Utility of D15 as carrier protein for the production of glycoconjugates.

To determine whether D15 may serve both as a protective antigen and a carrier, D15-PRP conjugation experiments were performed as described in Example 14. The D15-PRP conjugates were found to be highly immunogenic in rabbits and able to elicit both anti-D15 and anti-PRP IgG antibody responses as judged by D15-specific ELISA and PRP-BSA immunoassay (Table 9). These results clearly demonstrate the practical utility of D15 as a carrier protein for glycoconjugation technology.

In preferred embodiments of the present invention, the carrier function of D15 can be generally utilized to prepare chimeric molecules and conjugate vaccines against pathogenic bacteria, including encapsulated bacteria. Thus, the glycoconjugates of the present inventions may be applied to vaccinations to confer protection against infection with any bacteria having polysaccharide antigens, including, for example, Haemophilus influenzae, Streptococcus pneumoniae, Escherichia coli, Neisseria meningitidis, Salmonella typhi, Streptococcus mutans, Cryptococcus neoformans, Klebsiella, Staphylococcus aureus and Pseudomonas aeruginosa.

In another embodiment, the carrier function of D15 may be used, for example, to induce immunity toward abnormal polysaccharides of tumor cells, or to produce

rD15 protein and its fragments were found to cross-react immunologically with the native D15 antigen isolated from both typeable and non-typeable H. influenzae isolates and thus represent cross-reactive immunogens for inclusion in a vaccine against diseases caused by H. influenzae. Furthermore, Haemophilus convalescent serum recognized D15 purified from H. influenzae as described herein, rD15 and N-terminal rD15 fragment.

In another embodiment, the present invention provides a gene coding for the outer membrane protein D15 from H. influenzae having the specific nucleotide sequences described herein or ones substantially homologous thereto (i.e. those which hybridize under stringent conditions to such sequences), for genetically engineering hybrids or chimeric proteins containing a D15 fragment fused to another polypeptide or protein or a polysaccharide, such as H. influenzae outer membrane proteins, for example, P1, P2, or P6 or PRP. As a result, the hybrids, chimeric proteins or glycoconjugates may have higher protectivity against H. influenzae than D15, or P1, or P2, or P6, or PRP alone.

Thus, D15 outer membrane protein can function both as a protective antigen and as a carrier in a conjugate vaccine to provide autologous T-cell priming, wherein the hapten part of the conjugate is the capsular polysaccharide moiety (PRP) of H. influenzae. This D15-carbohydrate conjugate can elicit antibodies against both PRP and D15, and thus should enhance the level of protection against H. influenzae-related diseases, especially in infants.

In another embodiment, the present invention comprises an essentially pure form of at least one protein or peptide containing an amino acid sequence corresponding to at least one antigenic determinant of D15, which peptide is capable of eliciting polyclonal antibodies against H. influenzae in mammals. These D15-

catarrhalis, Staphylococcus aureus, or respiratory syncytial virus, in the presence or absence of adjuvant.

The D15 peptides (Table 2) or any portion, variant or mutant thereof, can easily be synthesized either manually or with a commercially available peptide synthesizer, such as the Applied Biosystems Model 430A synthesizer.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis, and treatment of diseases caused by Haemophilus infections, and the generation of immunological reagents. A further non-limiting discussion of such uses is further presented below.

1. Vaccine preparation and use

Immunogenic compositions, suitable for use as vaccines, may be prepared from immunogenic D15 outer membrane protein, fragments or analogs thereof and/or peptides corresponding to portions of D15 as disclosed herein. The vaccine elicits an immune response which produces antibodies, including anti-D15 outer membrane protein antibodies and antibodies against D15 that are opsonizing or bactericidal. Should the vaccinated subject be challenged by Haemophilus, the antibodies bind to the D15 outer membrane protein and thereby inactivate the bacterium. Opsonizing and bactericidal antibodies represent examples of antibodies useful in protection against disease.

Vaccines containing peptides are generally well known in the art, as exemplified by U.S. Patents 4,601,903; 4,599,231; 4,599,230; and 4,596,792; all of which references are incorporated herein by reference. As to any further reference to patents and references in this description, they are as well hereby incorporated by reference without any further notice to that effect. Vaccines may be prepared as injectables, as liquid

However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms of the D15 outer membrane protein, analog, fragment and/or peptides. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage of the vaccine may also depend on the route of administration and varies according to the size of the host.

The nucleic acid molecules encoding the D15 outer membrane protein of the present invention may also be used directly for immunization by administration of the DNA directly, for example, by injection for genetic immunization or by constructing a live vector, such as Salmonella, BCG, adenovirus, poxvirus or vaccinia. A discussion of some live vectors that have been used to carry heterologous antigens to the immune system are discussed in, for example, O'Hagan (1992). Processes for the direct injection of DNA into test subjects for genetic immunization are described in, for example, Ulman et al. (1993).

The use of peptides in vivo may first require their chemical modification since the peptides themselves may not have a sufficiently long serum and/or tissue half-life. Such chemically modified peptides are referred to herein as peptide analogs. The term peptide analog extends to any functional chemical equivalent of a peptide characterized by its increased stability and/or efficacy in vivo or in vitro in respect of the practice of the invention. The term peptide analog is also used herein to extend to any amino acid derivative of the peptides as described herein. Peptide analogs contemplated herein are produced by procedures that include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide synthesis and the use of

nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids.

2. Immunoassays

The D15 outer membrane protein, analog, fragment and/or peptides of the present invention are useful as antigens in immunoassays, including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known to the art for the detection of anti-bacterial, Haemophilus, D15 and/or peptide antibodies. In ELISA assays, the D15 outer membrane protein, fragment or analogs thereof and/or peptides corresponding to portions of D15 outer membrane protein are immobilized onto a selected surface, for example, a surface exhibiting a protein affinity, such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed D15 outer membrane protein, analog, fragment and/or peptides, a nonspecific protein, such as bovine serum albumin (BSA) or casein, that is known to be antigenically neutral with regard to the test sample may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus decreases the background caused by nonspecific bindings of antisera onto the surface. Normally, the peptides

Haemophilus and other bacteria that have genes encoding D15 outer membrane proteins.

The nucleotide sequences comprising the sequence encoding the D15 outer membrane protein of the present invention are useful for their ability to selectively form duplex molecules with complementary stretches of other D15 genes. Depending on the application, a variety of hybridization conditions may be employed to achieve varying degrees of selectivity of the probe toward the other D15 genes. For a high degree of selectivity, stringent conditions are used to form the duplexes, such as low salt and/or high temperature conditions, such as provided by 0.02 M to 0.15 M NaCl at temperatures of between about 50°C to 70°C. For some applications, less stringent hybridization conditions are required such as 0.15 M to 0.9 M salt, at temperatures ranging from between about 20°C to 55°C. Hybridization conditions can also be rendered more stringent by the addition of increasing amounts of formamide, to destabilize the hybrid duplex. Thus, particular hybridization conditions can be readily manipulated, and will generally be a method of choice depending on the desired results.

In a clinical diagnostic embodiment, the nucleic acid sequences of the D15 outer membrane protein genes of the present invention may be used in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of providing a detectable signal. In some diagnostic embodiments, an enzyme tag, such as urease, alkaline phosphatase or peroxidase, instead of a radioactive tag may be used. In the case of enzyme tags, colorimetric indicator substrates are known which can be employed to provide means visible to the human eye or

The pBR322 plasmid, or other microbial plasmid or phage must also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of its own proteins.

In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as a transforming vector in connection with these hosts. For example, the phage in lambda GEM™-11 may be utilized in making recombinant phage vectors which can be used to transform host cells, such as E. coli LE392.

Promoters commonly used in recombinant DNA construction include the β-lactamase (penicillinase) and lactose promoter systems and other microbial promoters, such as the T7 promoter system. Details concerning the nucleotide sequences of promoters are known, enabling a skilled worker to ligate them functionally with plasmid vectors. The particular promoter used generally is a matter of choice depending upon the desired results. Hosts that are appropriate for expression of the transferrin receptor genes, fragment analogs or variants thereof include E. coli, Bacillus, Haemophilus, Bordetella, fungi, yeast, or the baculovirus and poxvirus expression systems may be used.

In accordance with an aspect of this invention, it is preferred to make the D15 outer membrane protein, fragment or analog thereof by recombinant methods, particularly since the naturally occurring D15 protein as purified from culture of a species of Haemophilus may include undesired contaminants, including trace amounts of toxic materials. This problem can be avoided by using recombinantly produced D15 outer membrane protein in heterologous systems which can be isolated from the host in a manner to minimize toxins in the purified material. Particularly desirable hosts for expression in this regard include Gram positive bacteria which do not have

the invention. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations. Immunological and recombinant DNA methods may not be explicitly described in this disclosure but are well within the scope of those skilled in the art.

EXAMPLES

Methods of molecular genetics, protein biochemistry, and immunology used but not explicitly described in this disclosure and these EXAMPLES are amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1

This Example illustrates the cloning and sequencing of the D15 genes.

Genomic DNA was purified from the Haemophilus influenzae type b strain Ca by lysis of the bacteria with pronase and sodium dodecylsulphate followed by phenol extraction and isopropanol precipitation, according to Berns and Thomas, 1965. The DNA was then partially digested with EcoRI and the DNA fraction containing 6-10 kb fragments was isolated following electrophoresis in low-melting point agarose. These fragments were ligated into a lambda gt11 Amp1 vector (Thomas and Rossi, 1986) and cloned as a lysogen into E. coli strain BTA282. Recombinant clones were selected for their ampicillin resistance conferred by the vector. To identify clones producing H. influenzae type b antigen, the clones were replica-plated on nitrocellulose filters and duplicate colonies induced for expression by temperature switch to 42°C for 2 hours. Colonies were lysed by wetting the filters with 1% sodium dodecylsulphate (SDS). The filters were then placed into a chloroform-saturated atmosphere for 15 min. The filters were then assayed by colony radioimmuno-assay using a hyperimmune rabbit anti-H. influenzae type b antiserum absorbed with E. coli lysate

plasmid transformed into E. coli HB101. Recombinant bacteria were found to produce the expected M_r 80 kDa H. influenzae type b antigen when examined by Western blotting. The insert DNA was then characterised by restriction endonuclease mapping. A 2.8 kb HindIII-EcoRI fragment was subcloned into pUC19 to generate plasmid pUC19/D15, which was transformed into E. coli HB101. The recombinant bacteria expressed a M_r 80 kD protein recognized by D15-specific antibodies on Western blot analysis of E. coli lysates.

Plasmid DNA was prepared from two individual colonies of recombinant E. coli HB101 containing the pUC19/D15 plasmid using standard techniques. Oligonucleotide sequencing primers of 17-25 bases in length were synthesized on the ABI model 380B DNA Synthesizer and purified by chromatography using OPC cartridges obtained from Applied Biosystems Inc., and used in accordance with the manufactures recommendations. Samples were sequenced using the ABI model 370A DNA Sequencer and dye terminator chemistry according to manufacturers' protocols. This sequence analysis indicated that the D15 gene contains an open reading frame encoding for 789 amino acids, including a putative signal sequence (Figure 1). The derived amino acid sequence was found to contain the sequence of an internal peptide obtained by thrombin digestion of native D15 that had been chemically determined. The amino acid composition of D15 derived from the D15 gene sequence was comparable (within experimental error) to that of the native protein as determined by amino acid analysis.

Example 2

This Example illustrates the preparation of chromosomal DNA from Haemophilus influenzae strains Eagan, MinnA, SB33, and PAK 12085.

H. influenzae Eagan and PAK 12085 chromosomal DNAs were digested with Sau3A I (0.5 unit/10 μg DNA) at 37°C for 15 minutes and size-fractionated by agarose gel electrophoresis. Gel slices corresponding to DNA fragments of 15-23 kb were excised and DNA was electroeluted overnight in dialysis tubing containing 3 mL of TAE (40mM Tris-acetate, 1mM EDTA, pH 8.0) at 14V. The DNA was precipitated twice and resuspended in water before overnight ligation with EMBL3 BamH I arms (Promega). The ligation mixture was packaged using the Lambda in vitro packaging kit (Amersham) according to the manufacturer's instructions and plated onto E. coli NM539 cells. The library was titrated, then amplified and stored at 4°C under 0.3% chloroform.

MinnA chromosomal DNA (10 μg) was digested with Sau3A I (40 units) for 2, 4, and 6 minutes then size-fractionated on a 10-30% sucrose gradient in TNE (20mM Tris-HCl, 5mM NaCl, 1mM EDTA, pH 8.0). Fractions containing DNA fragments >5 kb were pooled and precipitated. In a second experiment, chromosomal DNA (2.6 μg) was digested with Sau3A I (4 units) for 1, 2, and 3 minutes and size- fractionated by preparative agarose gel electrophoresis. Gel slices containing DNA fragments of 10-20 kb were excised and DNA extracted by a standard freeze/thaw technique. The size-fractionated DNA from the two experiments was pooled for ligation with BamH I arms of EMBL3 (Promega). The ligation mix was packaged using the Gigapack II packaging kit (Amersham) and plated on E. coli LE392 cells. The library was titrated, then amplified and stored at 4°C under 0.3% chloroform.

SB33 chromosomal DNA (20 μg) was digested with Sau3A I (40 units) for 2, 4, or 6 minutes and size-fractionated on a 10-30% sucrose gradient in TNE (20mM Tris-HCl, 5mM NaCl, 1mM EDTA, pH 8.0). Fractions containing fragments >5 kb were pooled. In a second experiment, SB33

compared with the amino acid sequence of the D15 protein of H. influenzae type b Ca (Figure 3).

Example 5

This Example illustrates the expression of rD15 protein in E. coli.

A 2.8 kb fragment HindIII-EcoRI was subcloned into pUC19 and this pUC19/D15 plasmid was transformed into E. coli HB101. Upon induction, the positive clones expressed an 80 kDa protein which was recognized by D15-specific antisera on Western blot analysis. A HindIII-Pst I fragment was also subcloned into pUC19 and shown to express a 67 kDa protein. According to the restriction map, this 67 kDa protein corresponded to a C-terminal truncated D15 protein. On Western blot analysis, this truncated D15 was still recognized by the D15-specific antisera.

Plasmids to express the D15 gene of the non-typeable strain SB33 in E. coli were constructed. Plasmid JB-1042-5-1 containing the SB33 D15 gene and its flanking regions, was digested with EcoR I and Hind III and the 3kb D15 insert subcloned into pUC to give plasmid pRY-60-1 (Figure 4). Appropriate oligonucleotides were synthesized to restore the native D15 sequence between the ATG codon of the expression plasmid pT7-7 and the BsrF I site within the D15 gene. These oligonucleotides had the following sequence:

Nde

5'- TATGGCACCTTTTGTGGCAAAAGATATTCGTGTGGATGGTGTTCAAGGTG

ACCGTGGAAAACACCGTTTTCTATAAGCACACCTACCACAAGTTCCACTGAATCT

ACTTAGAATCAACAAACCGAGCAAGTTTACCTGTTCGTG - SEQ ID NO: 50

TGGTTGTTTAGGCTCGTTCAAATGGACAAGCACGGCC-5'- SEQ ID NO: 51

BsrF I

Plasmid pRY-60-1 was digested with EcoR I and BsrF I and the DNA fragment containing most of the D15 gene was purified. pUC was digested with EcoR I and Nde I and the

produced by transformed E. coli was isolated by affinity purification on glutathione agarose.

Example 7

This Example describes alternative expression systems for rD15.

The D15 gene or fragments thereof are also expressed in E. coli under the control of other regulated promoters. The D15 gene or fragments thereof are expressed in the absence of the leader peptide, or in other cloning systems where toxicity of D15 expression to the host is not problematic. The gene or fragments thereof are synthesized de novo or by employing the polymerase chain reaction using suitable primers. These genes are cloned into suitable cloning vectors or bacteriophage vectors in E. coli or other suitable hosts directly when toxicity can be avoided. Expression systems are Gram-positive bacteria (such as Bacillus species), pox virus, adenovirus, baculovirus, yeast, fungi, BCG or mammalian expression systems.

Example 8

This Example illustrates the protocol for extraction and purification of rD15 from E. coli expression system.

The cell pellet from a 250 mL culture, prepared as described in Example 5, was resuspended in 40 mL of 50 mM Tris, pH 8.0, and disrupted by sonication (3 x 10 min, 70% duty circle). The extract was centrifuged at 20,000 x *g* and the resulting pellet saved. The initial pellet was re-extracted with 40 mL of 50 mM Tris, 0.5% Triton X-100, 10 mM EDTA, pH 8.0. The suspension was then sonicated for 10 minutes at 70% duty circle. The extract was centrifuged at 300 x *g* for 5 minutes. The resulting supernatant was centrifuged again at 20,000 x *g* for 30 min and the resulting pellet was saved. The pellet was resuspended in 50 mM Tris, 0.5% Triton X-100, 10 mM EDTA, pH 8.0. The suspension was then mixed with PBS/ 8 M urea to a final urea concentration of 6 M. The solution was

This Example illustrates the procedure used for N-terminal rD15 fragment purification from GST using Glutathione-Sepharose 4B affinity chromatography.

A thrombin-digested GST-(D15 fragment) sample, prepared as described in Example 10, was loaded onto a Glutathione-Sepharose 4B column (2 mL) equilibrated with PBS containing 1% Triton X-100. The run-through of the column containing the N-terminal rD15 fragment was saved. After washing the column with 20 mL of PBS, the affinity column was regenerated by removing GST using 50 mM Tris-HCl buffer, pH 8.0, containing 5 mM glutathione. The purity of rD15 fragment was analysed by SDS-PAGE (Figure 9, lane 5). This N-terminal rD15 fragment contains amino acids 63-223 of the D15 protein as a result of cleavage at the spacious thrombin site shown in Figure 1A.

Example 12

This Example illustrates the protocol used for the purification of D15-specific polyclonal antibodies by affinity chromatography using GST-(D15 fragment) fusion protein.

The recombinant GST-(D15 fragment) fusion protein, prepared as described in Example 9, was conjugated to cyanogen bromide-activated Sepharose. The affinity column was then used to purify antibodies from a rabbit hyperimmune anti-H. influenzae type b antiserum. The affinity purified-antibodies were shown by immunoblotting to react with a 80 kDa component present in the lysates of E. coli transformed with pUC9/D15 and in the lysates of several typeable and nontypeable H. influenzae isolates. These results confirmed that the DNA segment encoding the D15 fragment of the fusion protein was part of the open reading frame of the D15 gene.

Similarly, antisera raised against the recombinant fusion protein (Example 9) or the purified N-terminal rD15 fragment (Example 11) reacted with the D15 protein produced by H. influenzae strains (Example 13).

in Example 17. The mean molecular size of the PRP molecules used for conjugation was determined as being approximately 20,000 Daltons. The conjugation was carried out without a linker molecule but may also be carried out with a linker molecule. A PRP/D15 molar ratio of approximately 7 was used to provide an excess of PRP hapten.

The PRP/rD15 conjugate was tested according to the protocol of Example 18 for immunogenicity in rabbits and elicited both primary and secondary anti-PRP IgG and anti-D15 antibody responses (Table 9). Rabbit anti-rD15-PRP antisera also strongly reacted with both native D15 and rD15 as judged by immunoblot analysis. These data indicate that rD15 can be used as a carrier protein in a conjugate vaccine. In addition, a rD15-PRP conjugate vaccine should ensure a more consistent protection against H. influenzae type b disease, particularly in infants, as a result of the additional homotypic protection provided by antibodies directed against the D15 protein.

Example 15

This Example describes the preparation of D15 peptides.

D15 peptides (Table 2) were synthesized using an ABI 430A peptide synthesizer and optimized t-Boc chemistry as described by the manufacturer, then cleaved from the resin by hydrofluoric acid (HF). The peptides were purified by reversed-phase high performance liquid chromatography (RP-HPLC) on a Vydac C4 semi-preparative column (1 x 30 cm) using a 15 to 55% acetonitrile gradient in 0.1% trifluoryl acetic acid (TFA) developed over 40 minutes at a flow rate of 2 mL/min. All synthetic peptides (Table 2) used in biochemical and immunological studies were >95% pure as judged by analytical HPLC. Amino acid composition analyses of

equilibrated with 0.2 M sodium phosphate buffer, pH 7.2, and eluted with the same buffer. Fractions were monitored for absorbance at 230 nm. The first major protein peak was pooled and concentrated in a Centriprep 30 to 2.2 mL. The amount of protein was determined using the Bio Rad protein assay, and was found to be 300 μg/mL. The presence of PRP in the protein conjugate fraction was confirmed by the Orcinol test.

Example 18

This Example describes the protocol used for the production of anti-PRP antisera in animals using rD15-PRP conjugates.

Rabbits were immunized intramuscularly with rD15-PRP conjugates (Example 14) (5 to 50 μg PRP equivalent) mixed with 3 mg $AlPO_4$ per mL, followed by two booster doses (half amount of the same immunogen) at 2 week intervals. Antisera were collected every 2 weeks after the first injection, heat-inactivated at 56°C for 30 minutes and stored at -20°C.

Example 19

This Example illustrates the reactivity between D15 peptides and anti-peptide and D15-specific antisera using D15-specific and peptide-specific ELISAs.

Microtiter wells (Nunc-Immunoplate, Nunc, Denmark) were coated with 200 ng of purified rD15 or 500 ng of individual peptides in 50 μL of coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6) for 16 hours at room temperature. The plates were then blocked with 0.1% (w/v) BSA in phosphate buffer saline (PBS) for 30 minutes at room temperature. Serially diluted antisera were added to the wells and incubated for 1 hour at room temperature. After removal of the antisera, the plates were washed five times with PBS containing 0.1% (w/v) Tween-20 and 0.1% (w/v) BSA. $F(ab')_2$ fragments from goat anti-rabbit, guinea pig, mouse, or human IgG antibodies conjugated to horseradish peroxidase (Jackson ImmunoResearch Labs Inc.,

the substrate tetramethylbenzidine (TMB) in H_2O_2 (ADI, Toronto). The reaction was stopped with 1N H_2SO_4 and the optical density measured at 450 nm using a Titretek Multiskan II (Flow Labs., Virginia). A standard anti-PRP antiserum of known titer was included as positive control. Assays were performed in triplicate, and the reactive titer of each antiserum was defined as the reciprocal of the dilution consistently showing a 2-fold increase in O.D. value over that obtained with the pre-immune serum (Table 9).

Example 21

This Example describes the protocol used for the production of D15-specific antisera using purified D15, rD15 or N-terminal rD15 fragment.

New Zealand White rabbits (Maple Lane) and guinea pigs (Charles River) were immunized intramuscularly (IM) with a 10 μg dose of either affinity-purified native D15 (Example 13), recombinant D15 (Example 8) or N-terminal rD15 fragment (Example 11) emulsified in Freund's complete adjuvant (Difco). Animals were boosted on day 28 with another 10 μg dose of affinity-purified D15 or rD15 or rD15 fragment emulsified in Freund's incomplete adjuvant and bled on day 42 via the marginal ear vein. D15-specific polyclonal antibodies were purified from this material as described in Example 12.

Example 22

This Example illustrates the protective activity of D15-specific antisera against H. influenzae type b challenge using the infant rat model of bacteremia.

Five-day old infant rats were inoculated subcutaneously (SC) on the dorsum with 0.15 mL of two different rabbit anti-N-terminal rD15 fragments. Pre-immune sera were used as negative controls. One day after immunization, the infant rats were injected intraperitoneally (IP) with 200 colony-forming units (cfu) of Haemophilus influenzae type *b* Minn A strain (0.1

supernatant added to further expand and maintain the viability of the peptide-specific T-cells. After a further 6 day-incubation, the cells were washed three times, each time with 200 μL of culture medium.

Each set of cultures was then stimulated with the corresponding concentrations (1, 10 and 100 μg per mL) of the peptide in the presence of 2 x 10^5 irradiated (1,500 rad) BALB/c spleen cells in a final volume of 200 μL of culture medium. Sixty μL of supernatant were then removed from each microculture. The supernatants from each triplicate cultures set were pooled. All supernatants were assayed for IL-2, Interleukin-4 and Interferon-gamma (IFN-γ). Detections of IL-2 and IL-4 were performed using murine IL-2 and IL-4 ELISA kits purchased from Endogen Inc. (MA, USA) respectively. Assay of IFN-γ was performed using a mouse IFN-γ ELISA kit supplied by Genzyme Corporation (MA, USA). Test culture supernatants were assayed at 1 in 5 dilution according to the manufacturers' instructions. The results obtained are set forth in Table 7.

Example 25

This Example describes the general procedure used for the production of murine D15-specific monoclonal antibodies.

BALB/c mice were immunized intraperitoneally with 20 to 50 μg of the N-terminal rD15 fragment (Example 11) emulsified in Freund's complete adjuvant. Two weeks later, the mice were given another injection of the same amount of immunogen in incomplete Freund's adjuvant (IFA). Three days before the fusion, the mice were boosted again with the same amount of immunogen in IFA. Hybridomas were produced by fusion of splenic lymphocytes from immunized mice with non-secreting Sp2/0 myeloma cells as previously described by Hamel et al. (1987). D15-specific hybridomas were cloned by sequential limiting dilutions and screened for anti-D15 monoclonal

TABLE 1

PROTECTIVE EFFECT OF PASSIVELY TRANSFERRED ANTI-N-TERMINAL RD15 FRAGMENT ANTIBODIES IN THE INFANT RAT MODEL OF BACTEREMIA[1]

Rabbit antisera	cfu/0.1 mL blood		p value
	Pre-immune	Post-immunization	
Rb#434	510 (6/6)[2]	6 (1/6)	<0.001
Rb#435	910 (4/4)	6 (1/4)	<0.001

[1] Five-day old infant rats were passively immunized with 0.15 mL of rabbit anti-N-terminal rD15 fragment s.c. One day later, the infant rats were challenged with 200 cfu of H. influenzae type b strain MinnA (0.1 mL, IP). The blood samples were taken from each rat 24 hours after the challenge and analysed for bacteria counts.

[2] The parentheses indicate the number of rats found to be bacteremic out of the total number of rats challenged.

D15-P29	619-646	LWVVSAKASAGYANGFGNKRLPFYQTYT	42
D15-P30	641-666	FYQTYTAGGIGSLRGFAYGSIGPNAI	43
D15-P31	662-688	GPNAIYAEYGNGSGTGTFKKISSDVIG	44
D15-P32	681-709	KISSDVIGGNAIATASAELIVPTPFVSDK	45
D15-P33	705-731	FVSDKSQNTVRTSLFVDAASVWNTKWK	46
D15-P34	725-750	VWNTKWKSDKNGLESDVLKRLPDYGK	47
D15-P35	745-771	LPDYGKSSRIRASTGVGFQWQSPIGPL	48
D15-P36	769-798	GPLVFSYAKPIKKYENDDVEQFQFSIGGSF	49

TABLE 4

INHIBITION OF ANTI-N-TERMINAL rD15 FRAGMENT ANTIBODY-INDUCED PROTECTION BY D15 PEPTIDES IN THE INFANT RAT MODEL OF BACTEREMIA

Group #	Antibody	cfu / 10 μL blood	cfu in each group/ cfu in group #4 (control) (%)
1	Anti-D15 Ab + PBS	60 ± 120 (3/7)	3
2	Anti-D15 Ab + peptides	300 ± 240 (6/7)	13
3	Anti-D15 Ab + rD15	1,520 ± 1,280 (7/7)	64
4	PBS + peptides	2,360 ± 1,200 (6/7)	100

One half mL of rabbit anti-N-terminal rD15 fragment antiserum (Anti-rD15 fragment Ab) was mixed with either nine D15 peptides (100 μg of peptides D15-P2 to D15-P10, See TABLE 2) or with 600 μg of N-terminal rD15 fragment at room temperature for 1 hr. Antiserum and peptides mixed with PBS were used as controls. Seven-day old infant rats were injected s.c. with 0.2 mL of the various preparations. After 24 h, the infant rats were challenged I.P. with 200 cfu of *H. influenzae* type b strain MinnA. The blood samples were taken at 24 h after the challenge. The numbers in parentheses indicate the number of animals that were bacteremic out of the total number of animals challenged. The level of bacteremia is expressed as the mean of values obtained from seven infant rats tested individually ± one standard deviation (SD).

TABLE 6

REACTIVITY OF RABBIT, GUINEA PIG AND MOUSE ANTI-rD15 ANTISERA WITH D15 PEPTIDES

Peptide	Reactive Titer[1] Rabbit[2]	Guinea Pig[3]	Mouse[4]
D15-P1	-	-	+
D15-P2	-	+++	+
D15-P3	-	-	+
D15-P4	+	+	+
D15-P5	-	-	+
D15-P6	-	+	+
D15-P7	-	-	+
D15-P8	-	++++	++++
D15-P9	-	-	+
D15-P10	-	-	+++
D15-P11	-	-	+++
D15-P12	-	-	+
D15-P13	-	-	+
D15-P14	+++	+	+
D15-P15	-	-	+
D15-P16	-	-	+
D15-P17	-	-	+
D15-P18	-	-	+
D15-P19	-	-	+
D15-P20	-	-	+
D15-P21	-	-	+
D15-P22	-	-	+
D15-P23	-	-	+
D15-P24	-	-	+
D15-P25	-	-	+
D15-P26	-	-	+++
D15-P27	-	+	+

TABLE 7

T-CELL STIMULATORY ACTIVITY OF D15 PEPTIDES

Peptide	CYTOKINE RELEASE (pg/mL)[1] IL-2[2]	γ-IFN[3]	IL-4[4]
D15-P1	-	-	-
D15-P2	122	-	-
D15-P3	25	-	-
D15-P4	-	-	-
D15-P5	*742*	*38,000*	*13*
D15-P6	-	-	-
D15-P7	-	-	-
D15-P8	-	-	-
D15-P9	-	-	-
D15-P10	108	1,900	-
D15-P11	-	-	-
D15-P12	1,052	6,100	-
D15-P13	*105*	*6,200*	*56*
D15-P14	-	-	-
D15-P15	-	-	-
D15-P16	48	-	-
D15-P17	-	-	-
D15-P18	32	4,800	-
D15-P19	882	24,500	-
D15-P20	-	-	-
D15-P21	-	-	-
D15-P22	-	-	-
D15-P23	78	-	-
D15-P24	103	-	-
D15-P25	-	-	-
D15-P26	572	6,700	-
D15-P27	*274*	*7,505*	*68*

TABLE 8

RABBIT AND GUINEA PIG ANTIBODY RESPONSES TO D15 PEPTIDES

	Peptide-specific ELISAs	
	Reactive Titer[1]	
Immunogen	Rabbit[2]	Guinea Pig[3]
D15-P1	102,400	819,200
D15-P2	204,800	1,637,400
D15-P3	51,200	1,637,400
D15-P4	204,800	819,200
D15-P5	51,200	1,637,400
D15-P6	51,200	409,600
D15-P7	204,800	819,200
D15-P8	51,200	409,600
D15-P9	102,400	409,600
D15-P10	102,400	819,200
D15-P11	51,200	819,200
D15-P12	102,400	204,800
D15-P13	NT[4]	204,800
D15-P14	NT	409,600
D15-P15	NT	204,800
D15-P16	NT	819,200
D15-P17	NT	204,800
D15-P18	NT	312,500
D15-P19	NT	312,500
D15-P20	NT	62,500
D15-P21	NT	62,500
D15-P22	NT	12,500
D15-P23	NT	1,562,500
D15-P24	NT	312,500
D15-P25	NT	62,500

TABLE 9

RABBIT IgG ANTIBODY RESPONSE TO D15-PRP CONJUGATE

Rabbit[1] #	Reactive Titer Against[2]			
	PRP		rD15	
	2 doses	3 doses	2 doses	3 doses
489-1	1,600	3,200	1,600	6,400
490-1	1,600	1,600	6,400	25,600

[1] Rabbits were immunized intramuscularly with rD15-PRP conjugates (5 to 50 μg PRP equivalent) mixed with 3 mg $ALPO_4$ per mL, followed by two booster doses (half amount of the same immunogen) at 2 week intervals.

[2] Reactive titres is based on PRP specific and D-15 specific ELISAs.

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8. The recombinant vector of claim 6 wherein said DNA segment encodes a polypeptide of at least 6 residues.

9. The recombinant vector of claim 8 wherein said polypeptide is selected from those shown in Table 2.

10. The recombinant vector of claim 6, 7, 8 or 9 wherein said DNA segment consists of no more than the coding sequence for said D15 outer membrane protein.

11. The recombinant vector of claim 10, wherein the DNA segment further comprises a nucleic acid sequence encoding a leader sequence for export of said gene product from said host.

12. A purified and isolated protein encoded by the DNA fragment contained in the recombinant vector of claim 10 or 11.

13. A purified and isolated D15 outer membrane protein, or a portion thereof.

14. The protein of claim 13 wherein the D15 outer membrane protein is a Haemophilus D15 outer membrane protein.

15. The protein of claim 14 wherein the D15 outer membrane protein is a Haemophilus influenzae D15 outer membrane protein.

16. The protein of claim 15 wherein the Haemophilus influenzae is a type b Haemophilus influenzae strain.

17. The protein of claim 16 wherein the Haemophilus influenzae type b strain is selected from Ca, MinnA and Eagan strains.

18. The protein of claim 15 wherein the Haemophilus influenzae is a non-typeable Haemophilus influenzae strain.

19. The protein of claim 18 wherein the non-typeable Haemophilus influenzae strain is selected from PAK12085 and SB33 strains.

20. A synthetic peptide containing an amino acid sequence corresponding to the amino acid sequence of the protein or portion thereof claimed in any one of claims

30. The chimeric molecule of claim 29 wherein said another polypeptide or protein comprises a P1, P2 or P6 outer membrane protein of H. influenzae.

31. The chimeric molecule of claim 28 wherein said polysaccharide comprises a PRP molecule from H. influenzae.

FIG.1A.

H. influenzae b Ca strain D15 sequence

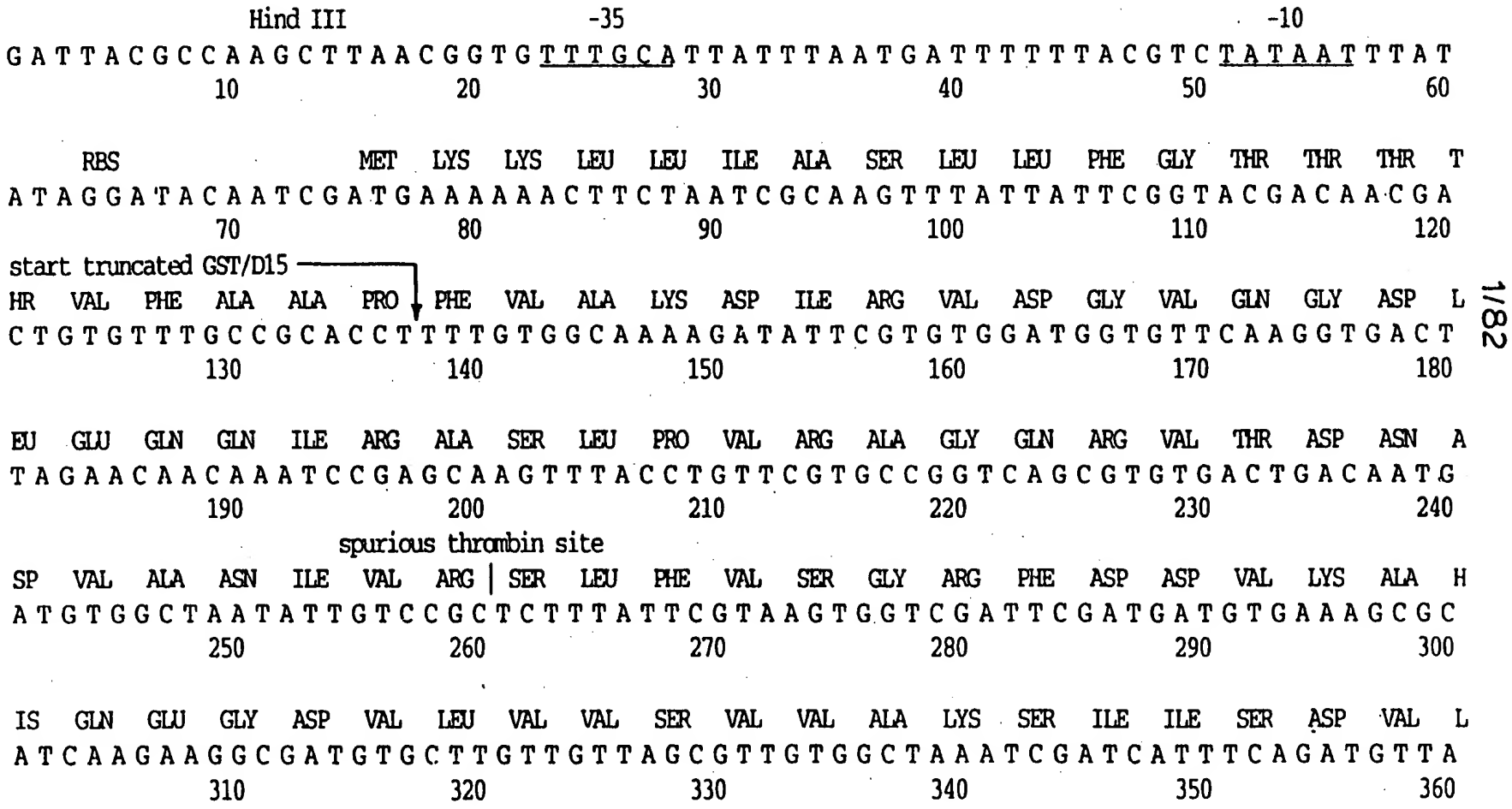


FIG.1A.(CONTINUED)

```
YS  ILE  LYS  GLY  ASN  SER  VAL  ILE  PRO  THR  GLU  ALA  LEU  LYS  GLN  ASN  LEU  ASP  ALA  ASN  G
AAATCAAAGGTAACTCTGTTATTCCCACTGAAGCACTTAAACAAAACTTAGATGCTAACG
            370            380            390            400            410            420

LY  PHE  LYS  VAL  GLY  ASP  VAL  LEU  ILE  ARG  GLU  LYS  LEU  ASN  GLU  PHE  ALA  LYS  SER  VAL  L
GGTTTAAAGTTGGCGATGTTTTAATTCGAGAAAAATTAAATGAATTTGCCAAAAGTGTAA
            430            440            450            460            470            480

YS  GLU  HIS  TYR  ALA  SER  VAL  GLY  ARG  TYR  ASN  ALA  THR  VAL  GLU  PRO  ILE  VAL  ASN  THR  L
AAGAGCACTATGCAAGTGTAGGTCGCTATAACGCAACAGTTGAACCTATTGTCAATACGC
            490            500            510            520            530            540

EU  PRO  ASN  ASN  ARG  ALA  GLU  ILE  LEU  ILE  GLN  ILE  ASN  GLU  ASP  ASP  LYS  ALA  LYS  LEU  A
TACCAAATAATCGCGCTGAAATTTTAATTCAAATCAATGAAGATGATAAAGCAAAAATTGG
            550            560            570            580            590            600

LA  SER  LEU  THR  PHE  LYS  GLY  ASN  GLU  SER  VAL  SER  SER  SER  THR  LEU  GLN  GLU  GLN  MET  G
CATCATTAACTTTCAAGGGGAACGAATCTGTTAGTAGCAGTACATTACAAGAACAAATGG
            610            620            630            640            650            660

LU  LEU  GLN  PRO  ASP  SER  TRP  TRP  LYS  LEU  TRP  GLY  ASN  LYS  PHE  GLU  GLY  ALA  GLN  PHE  G
AATTACAACCTGATTCTTGGTGGAAATTATGGGGAAATAAATTTGAAGGTGCGCAATTCG
            670            680            690            700            710            720

  end truncated GST/D15 ——————————————————▼
LU  LYS _ ASP  LEU  GLN  SER  ILE  ARG  ASP  TYR  TYR  LEU  ASN  ASN  GLY  TYR  ALA  LYS  ALA  GLN  I
AGAAAGATTTGCAGTCAATTCGTGATTATTATTTAAATAATGGCTATGCCAAAGCACAAA
            730            740            750            760            770            780
```

FIG.1A.(CONTINUED)

```
LE  THR  LYS  THR  ASP  VAL  GLN  LEU  ASN  ASP  GLU  LYS  THR  LYS  VAL  ASN  VAL  THR  ILE  ASP  V
TTACTAAAACGGATGTTCAGCTAAATGATGAAAAAACAAAAGTTAATGTAACCATTGATG
            790                 800                 810                 820                 830                 840

AL  ASN  GLU  GLY  LEU  GLN  TYR  ASP  LEU  ARG  SER  ALA  ARG  ILE  ILE  GLY  ASN  LEU  GLY  GLY  M
TAAATGAAGGTTTACAGTATGACCTTCGTAGTGCACGCATTATAGGTAATCTGGGAGGTA
            850                 860                 870                 880                 890                 900

ET  SER  ALA  GLU  LEU  GLU  PRO  LEU  LEU  SER  ALA  LEU  HIS  LEU  ASN  ASP  THR  PHE  ARG  ARG  S
TGTCTGCCGAGCTTGAACCTTTACTTTCAGCATTACATTTAAATGATACTTTCCGCCGTA
            910                 920                 930                 940                 950                 960

ER  ASP  ILE  ALA  ASP  VAL  GLU  ASN  ALA  ILE  LYS  ALA  LYS  LEU  GLY  GLU  ARG  GLY  TYR  GLY  S
GTGATATTGCAGATGTAGAAAATGCAATTAAAGCAAAACTTGGAGAACGCGGTTACGGTA
            970                 980                 990                 1000                1010                1020

ER  ALA  THR  VAL  ASN  SER  VAL  PRO  ASP  PHE  ASP  ASP  ALA  ASN  LYS  THR  LEU  ALA  ILE  THR  L
GCGCAACGGTAAATTCAGTACCTGATTTTGATGATGCAAATAAAACATTAGCGATAACCC
            1030                1040                1050                1060                1070                1080

EU  VAL  VAL  ASP  ALA  GLY  ARG  ARG  LEU  THR  VAL  ARG  GLN  LEU  ARG  PHE  GLU  GLY  ASN  THR  V
TTGTTGTTGATGCTGGACGACGTTTAACTGTTCGCCAACTTCGCTTTGAAGGAAATACCG
            1090                1100                1110                1120                1130                1140
```

FIG.1A.(CONTINUED)

```
AL  SER  ALA  ASP  SER  THR  LEU  ARG  GLN  GLU  MET  ARG  GLN  GLN  GLU  GLY  THR  TRP  TYR  ASN  S
TTTCTGCTGATAGCACTTTACGTCAGGAAATGCGCCAACAAGAAGGAACTTGGTATAATT
            1150          1160          1170          1180          1190          1200

ER  GLN  LEU  VAL  GLU  LEU  GLY  LYS  ILE  ARG  LEU  ASP  ARG  THR  GLY  PHE  PHE  GLU  THR  VAL  G
CACAATTAGTTGAGTTAGGAAAAATTCGCTTAGATCGTACAGGTTTCTTCGAAACAGTCG
            1210          1220          1230          1240          1250          1260

LU  ASN  ARG  ILE  ASP  PRO  ILE  ASN  GLY  SER  ASN  ASP  GLU  VAL  ASP  VAL  VAL  TYR  LYS  VAL  L
AAAACCGAATTGATCCTATCAATGGTAGTAATGATGAAGTGGATGTCGTATATAAAGTCA
            1270          1280          1290          1300          1310          1320

YS  GLU  ARG  ASN  THR  GLY  SER  ILE  ASN  PHE  GLY  ILE  GLY  TYR  GLY  THR  GLU  SER  GLY  ILE  S
AAGAACGTAACACGGGTAGTATCAACTTTGGTATTGGTTACGGTACAGAGAGTGGTATTA
            1330          1340          1350          1360          1370          1380

ER  TYR  GLN  ALA  SER  VAL  LYS  GLN  ASP  ASN  PHE  LEU  GLY  THR  GLY  ALA  ALA  VAL  SER  ILE  A
GTTATCAAGCAAGTGTTAAACAAGATAATTTCTTGGGAACAGGGGCGGCAGTAAGTATAG
            1390          1400          1410          1420          1430          1440

LA  GLY  THR  LYS  ASN  ASP  TYR  GLY  THR  SER  VAL  ASN  LEU  GLY  TYR  THR  GLU  PRO  TYR  PHE  T
CTGGTACGAAAAATGATTATGGTACGAGTGTCAATTTGGGTTATACCGAGCCCTATTTTA
            1450          1460          1470          1480          1490          1500

HR  LYS  ASP  GLY  VAL  SER  LEU  GLY  GLY  ASN  VAL  PHE  PHE  GLU  ASN  TYR  ASP  ASN  SER  LYS  S
CTAAAGATGGTGTAAGTCTTGGTGGAAATGTTTTCTTTGAAAACTACGATAACTCTAAAA
            1510          1520          1530          1540          1550          1560
```

FIG.1A.(CONTINUED)

ER ASP THR SER SER ASN TYR LYS ARG THR THR TYR GLY SER ASN VAL THR LEU GLY PHE P
G T G A T A C A T C C T C T A A C T A T A A G C G T A C G A C T T A C G G A A G T A A T G T T A C T T T A G G T T T C C
1570 1580 1590 1600 1610 1620

RO VAL ASN GLU ASN ASN SER TYR TYR VAL GLY LEU GLY HIS THR TYR ASN LYS ILE SER A
C T G T A A A T G A A A A T A A C T C C T A T T A T G T A G G A T T A G G T C A T A C C T A T A A T A A A A T T A G T A
1630 1640 1650 1660 1670 1680

SN PHE ALA LEU GLU TYR ASN ARG ASN LEU TYR ILE GLN SER MET LYS PHE LYS GLY ASN G
A C T T T G C T C T A G A A T A T A A C C G T A A T T T A T A T A T T C A A T C A A T G A A A T T T A A A G G T A A T G
1690 1700 1710 1720 1730 1740

LY ILE LYS THR ASN ASP PHE ASP PHE SER PHE GLY TRP ASN TYR ASN SER LEU ASN ARG G
G C A T T A A A A C A A A T G A C T T T G A T T T T T C T T T T G G T T G G A A C T A T A A C A G C C T T A A T A G A G
1750 1760 1770 1780 1790 1800

LY TYR PHE PRO THR LYS GLY VAL LYS ALA SER LEU GLY GLY ARG VAL THR ILE PRO GLY S
G C T A T T T C C C A A C T A A A G G G G T T A A A G C A A G T C T T G G T G G A C G A G T T A C T A T T C C A G G T T
1810 1820 1830 1840 1850 1860

ER ASP ASN LYS TYR TYR LYS LEU SER ALA ASP VAL GLN GLY PHE TYR PRO LEU ASP ARG A
C T G A T A A C A A A T A C T A C A A A C T A A G T G C A G A T G T A C A G G G T T T C T A C C C A T T A G A C A G A G
1870 1880 1890 1900 1910 1920

SP HIS LEU TRP VAL VAL SER ALA LYS ALA SER ALA GLY TYR ALA ASN GLY PHE GLY ASN L
A T C A C C T C T G G G T T G T A T C T G C A A A A G C A T C T G C A G G A T A T G C A A A T G G T T T T G G A A A C A
1930 1940 1950 1960 1970 1980

FIG.1A.(CONTINUED)

```
YS  ARG  LEU  PRO  PHE  TYR  GLN  THR  TYR  THR  ALA  GLY  GLY  ILE  GLY  SER  LEU  ARG  GLY  PHE  A
AGCGTTTACCGTTCTATCAAACTTATACAGCGGGTGGCATCGGTTCATTACGTGGTTTTG
          1990              2000              2010              2020              2030              2040

LA  TYR  GLY  SER  ILE  GLY  PRO  ASN  ALA  ILE  TYR  ALA  GLU  TYR  GLY  ASN  GLY  SER  GLY  THR  G
CTTATGGTAGTATTGGACCTAACGCAATTTATGCCGAATATGGTAATGGTAGTGGTACTG
          2050              2060              2070              2080              2090              2100

LY  THR  PHE  LYS  LYS  ILE  SER  SER  ASP  VAL  ILE  GLY  GLY  ASN  ALA  ILE  ALA  THR  ALA  SER  A
GTACTTTTAAGAAGATAAGTTCTGATGTGATTGGTGGTAATGCAATCGCTACAGCTAGCG
          2110              2120              2130              2140              2150              2160

LA  GLU  LEU  ILE  VAL  PRO  THR  PRO  PHE  VAL  SER  ASP  LYS  SER  GLN  ASN  THR  VAL  ARG  THR  S
CAGAGTTAATTGTGCCAACTCCATTTGTGAGCGATAAGAGCCAAAATACGGTCCGAACCT
          2170              2180              2190              2200              2210              2220

ER  LEU  PHE  VAL  ASP  ALA  ALA  SER  VAL  TRP  ASN  THR  LYS  TRP  LYS  SER  ASP  LYS  ASN  GLY  L
CCTTATTTGTTGATGCGGCAAGTGTTTGGAATACTAAATGGAAATCAGATAAAAATGGAT
          2230              2240              2250              2260              2270              2280

EU  GLU  SER  ASP  VAL  LEU  LYS  ARG  LEU  PRO  ASP  TYR  GLY  LYS  SER  SER  ARG  ILE  ARG  ALA  S
TAGAGAGCGATGTATTAAAAAGATTGCCTGATTATGGCAAATCAAGCCGTATTCGCGCCT
          2290              2300              2310              2320              2330              2340
```

FIG.1A.(CONTINUED)

```
ER  THR  GLY  VAL  GLY  PHE  GLN  TRP  GLN  SER  PRO  ILE  GLY  PRO  LEU  VAL  PHE  SER  TYR  ALA  L
CTACAGGTGTCGGATTCCAATGGCAATCTCCTATTGGGCCATTGGTATTCTCTTATGCCA
          2350          2360          2370          2380          2390          2400

YS  PRO  ILE  LYS  LYS  TYR  GLU  ASN  ASP  ASP  VAL  GLU  GLN  PHE  GLN  PHE  SER  ILE  GLY  GLY  S
AACCAATTAAAAAAATATGAAAATGATGATGTCGAACAGTTCCAATTTAGTATTGGAGGTT
          2410          2420          2430          2440          2450          2460

ER  PHE  ***  ***
CTTTCTAATAAATTGAACTTTTTTCTTCATCAGAACTCAAAAACAACGTTCTCTGCCTAA
          2470          2480          2490          2500          2510          2520

TTTAATTGGGCAGAGAAAATATTAAACCCATCATTTAATTAAGGATATTTATCAAATGAA
          2530          2540          2550          2560          2570          2580

AAACATCGCAAAAGTAACCGCACTTGCTTTAGGTATTGCACTTGCTTCAGGCTATGCTTC
          2590          2600          2610          2620          2630          2640

CGCTGAAGAAAAAATTGCTTTCATTAATGCAGGTATATTTTTCAACATCACCCAGATCGC
          2650          2660          2670          2680          2690          2700

CAAGCGGTAGCAGATAAACTTGATGCTGAATTTAAACCTGTAGCTGAGAAATTAGCAGCA
          2710          2720          2730          2740          2750          2760
```

FIG.1A.(CONTINUED)

```
AGCAAAAAAGAAGTTGATGATAAAATTGCTGCTGCTCGTAAAAAAGTAGAAGCAAAAGTT
          2770          2780          2790          2800          2810          2820

GCGGCTTTAGAAAAAGATGCACCTCGCTTACGTCAAGCTGATATTCAAAAACGCCAACAG
          2830          2840          2850          2860          2870          2880

GAGATTAATAAATTAGGTGCGGCTGAAGATGCTGAATTACAAAAATTAATGCAAGAACAA
          2890          2900          2910          2920          2930          2940

GATAAAAAA
```

FIG.1B.

DS-712-2-1 DNA, Eagan D15 sequence
IS THE SEQUENCE BEING TRANSLATED

```
ACAGGACAGCTTTCCCTTTTAACCTTGAAAATATTAGGGAAATTACTTCCTGGCGATTTG
          10        20        30        40        50        60

TCATTAAATAATTTAAGTGGGCCAATTTCTATTGCAAAAGGTGCTGGCCCATCAGCAAAT
          70        80        90       100       110       120

ATTGGATTGGTGTATTTTTTAAGTTTTATGGCACTGATTAGTGTAAATTTAGGGATTATG
         130       140       150       160       170       180

AATTTATTTCCATTACCAGTATTAGATGGCGGTCATTTAGTTTTTTTTAACAATGGAAGCT
         190       200       210       220       230       240

GTTAAAGGAAAACCTGTTTCTGAGCGGGTGCAAAGCATCTGTTATCGAATTGGCGCAGCA
         250       260       270       280       290       300

CTGTTATTAAGCTTAACGGTGTTTGCATTATTTAATGATTTTTTACGTCTATAATTTATA
         310       320       330       340       350       360
```

FIG.1B.(CONTINUED)

```
                        MET  LYS  LYS  LEU  LEU  ILE  ALA  SER  LEU  LEU  PHE  GLY  THR  THR  THR  THR
TAGGATACAATCGATGAAAAAACTTCTAATCGCAAGTTTATTATTCGGTACGACAACGAC
            370                  380                  390                  400                  410                  420

  VAL  PHE  ALA  ALA  PRO  PHE  VAL  ALA  LYS  ASP  ILE  ARG  VAL  ASP  GLY  VAL  GLN  GLY  ASP  LEU
TGTGTTTGCCGCACCTTTTGTGGCAAAAGATATTCGTGTGGATGGTGTTCAAGGTGACTT
            430                  440                  450                  460                  470                  480

  GLU  GLN  GLN  ILE  ARG  ALA  SER  LEU  PRO  VAL  ARG  ALA  GLY  GLN  ARG  VAL  THR  ASP  ASN  ASP
AGAACAACAAATCCGAGCAAGTTTACCTGTTCGTGCCGGTCAGCGTGTGACTGACAATGA
            490                  500                  510                  520                  530                  540

  VAL  ALA  ASN  ILE  VAL  ARG  SER  LEU  PHE  VAL  SER  GLY  ARG  PHE  ASP  ASP  VAL  LYS  ALA  HIS
TGTGGCTAATATTGTCCGCTCTTTATTCGTAAGTGGTCGATTCGATGATGTGAAAGCGCA
            550                  560                  570                  580                  590                  600

  GLN  GLU  GLY  ASP  VAL  LEU  VAL  VAL  SER  VAL  VAL  ALA  LYS  SER  ILE  ILE  SER  ASP  VAL  LYS
TCAAGAAGGCGATGTGCTTGTTGTTAGCGTTGTGGCTAAATCGATCATTTCAGATGTTAA
            610                  620                  630                  640                  650                  660

  ILE  LYS  GLY  ASN  SER  VAL  ILE  PRO  THR  GLU  ALA  LEU  LYS  GLN  ASN  LEU  ASP  ALA  ASN  GLY
AATCAAAGGTAACTCTGTTATTCCCACTGAAGCACTTAAACAAAACTTAGATGCTAACGG
            670                  680                  690                  700                  710                  720
```

FIG.1B.(CONTINUED)

```
   PHE  LYS  VAL  GLY  ASP  VAL  LEU  ILE  ARG  GLU  LYS  LEU  ASN  GLU  PHE  ALA  LYS  SER  VAL  LYS
G T T T A A A G T T G G C G A T G T T T T A A T T C G A G A A A A A T T A A A T G A A T T T G C C A A A A G T G T A A A
               730                  740                  750                  760                  770                  780

   GLU  HIS  TYR  ALA  SER  VAL  GLY  ARG  TYR  ASN  ALA  THR  VAL  GLU  PRO  ILE  VAL  ASN  THR  LEU
A G A G C A C T A T G C A A G T G T A G G T C G C T A T A A C G C A A C A G T T G A A C C T A T T G T C A A T A C G C T
               790                  800                  810                  820                  830                  840

   PRO  ASN  ASN  ARG  ALA  GLU  ILE  LEU  ILE  GLN  ILE  ASN  GLU  ASP  ASP  LYS  ALA  LYS  LEU  ALA
A C C A A A T A A T C G C G C T G A A A T T T T A A T T C A A A T C A A T G A A G A T G A T A A A G C A A A A T T G G C
               850                  860                  870                  880                  890                  900

   SER  LEU  THR  PHE  LYS  GLY  ASN  GLU  SER  VAL  SER  SER  SER  THR  LEU  GLN  GLU  GLN  MET  GLU
A T C A T T A A C T T T C A A G G G G A A C G A A T C T G T T A G T A G C A G T A C A T T A C A A G A A C A A A T G G A
               910                  920                  930                  940                  950                  960

   LEU  GLN  PRO  ASP  SER  TRP  TRP  LYS  LEU  TRP  GLY  ASN  LYS  PHE  GLU  GLY  ALA  GLN  PHE  GLU
A T T A C A A C C T G A T T C T T G G T G G A A A T T A T G G G G A A A T A A A T T T G A A G G T G C G C A A T T C G A
               970                  980                  990                  1000                 1010                 1020

   LYS  ASP  LEU  GLN  SER  ILE  ARG  ASP  TYR  TYR  LEU  ASN  ASN  GLY  TYR  ALA  LYS  ALA  GLN  ILE
G A A A G A T T T G C A G T C A A T T C G T G A T T A T T A T T T A A A T A A T G G C T A T G C C A A A G C A C A A A T
               1030                 1040                 1050                 1060                 1070                 1080

   THR  LYS  THR  ASP  VAL  GLN  LEU  ASN  ASP  GLU  LYS  THR  LYS  VAL  ASN  VAL  THR  ILE  ASP  VAL
T A C T A A A A C G G A T G T T C A G C T A A A T G A T G A A A A A A C A A A A G T T A A T G T A A C C A T T G A T G T
               1090                 1100                 1110                 1120                 1130                 1140
```

FIG.1B.(CONTINUED)

```
   ASN  GLU  GLY  LEU  GLN  TYR  ASP  LEU  ARG  SER  ALA  ARG  ILE  ILE  GLY  ASN  LEU  GLY  GLY  MET
AAATGAAGGTTTACAGTATGACCTTCGTAGTGCACGCATTATAGGTAATCTGGGAGGTAT
          1150         1160         1170         1180         1190         1200

   SER  ALA  GLU  LEU  GLU  PRO  LEU  LEU  SER  ALA  LEU  HIS  LEU  ASN  ASP  THR  PHE  ARG  ARG  SER
GTCTGCCGAGCTTGAACCTTTACTTTCAGCATTACATTTAAATGATACTTTCCGCCGTAG
          1210         1220         1230         1240         1250         1260

   ASP  ILE  ALA  ASP  VAL  GLU  ASN  ALA  ILE  LYS  ALA  LYS  LEU  GLY  GLU  ARG  GLY  TYR  GLY  SER
TGATATTGCAGATGTAGAAAATGCAATTAAAGCAAAACTTGGAGAACGCGGTTACGGTAG
          1270         1280         1290         1300         1310         1320

   ALA  THR  VAL  ASN  SER  VAL  PRO  ASP  PHE  ASP  ASP  ALA  ASN  LYS  THR  LEU  ALA  ILE  THR  LEU
CGCAACGGTAAATTCAGTACCTGATTTTGATGATGCAAATAAAACATTAGCGATAACCCT
          1330         1340         1350         1360         1370         1380

   VAL  VAL  ASP  ALA  GLY  ARG  ARG  LEU  THR  VAL  ARG  GLN  LEU  ARG  PHE  GLU  GLY  ASN  THR  VAL
TGTTGTTGATGCTGGACGACGTTTAACTGTTCGCCAACTTCGCTTTGAAGGAAATACCGT
          1390         1400         1410         1420         1430         1440

   SER  ALA  ASP  SER  THR  LEU  ARG  GLN  GLU  MET  ARG  GLN  GLN  GLU  GLY  THR  TRP  TYR  ASN  SER
TTCTGCTGATAGCACTTTACGTCAGGAAATGCGCCAACAAGAAGGAACTTGGTATAATTC
          1450         1460         1470         1480         1490         1500
```

FIG.1B.(CONTINUED)

```
   GLN  LEU  VAL  GLU  LEU  GLY  LYS  ILE  ARG  LEU  ASP  ARG  THR  GLY  PHE  PHE  GLU  THR  VAL  GLU
A C A A T T A G T T G A G T T A G G A A A A A T T C G C T T A G A T C G T A C A G G T T T C T T C G A A A C A G T C G A
             1510              1520              1530              1540              1550              1560

   ASN  ARG  ILE  ASP  PRO  ILE  ASN  GLY  SER  ASN  ASP  GLU  VAL  ASP  VAL  VAL  TYR  LYS  VAL  LYS
A A A C C G A A T T G A T C C T A T C A A T G G T A G T A A T G A T G A A G T G G A T G T C G T A T A T A A A G T C A A
             1570              1580              1590              1600              1610              1620

   GLU  ARG  ASN  THR  GLY  SER  ILE  ASN  PHE  GLY  ILE  GLY  TYR  GLY  THR  GLU  SER  GLY  ILE  SER
A G A A C G T A A C A C G G G T A G T A T C A A C T T T G G T A T T G G T T A C G G T A C A G A G A G T G G T A T T A G
             1630              1640              1650              1660              1670              1680

   TYR  GLN  ALA  SER  VAL  LYS  GLN  ASP  ASN  PHE  LEU  GLY  THR  GLY  ALA  ALA  VAL  SER  ILE  ALA
T T A T C A A G C A A G T G T T A A A C A A G A T A A T T T C T T G G G A A C A G G G G C G G C A G T A A G T A T A G C
             1690              1700              1710              1720              1730              1740

   GLY  THR  LYS  ASN  ASP  TYR  GLY  THR  SER  VAL  ASN  LEU  GLY  TYR  THR  GLU  PRO  TYR  PHE  THR
T G G T A C G A A A A A T G A T T A T G G T A C G A G T G T C A A T T T G G G T T A T A C C G A G C C C T A T T T T A C
             1750              1760              1770              1780              1790              1800

   LYS  ASP  GLY  VAL  SER  LEU  GLY  GLY  ASN  VAL  PHE  PHE  GLU  ASN  TYR  ASP  ASN  SER  LYS  SER
T A A A G A T G G T G T A A G T C T T G G T G G A A A T G T T T T C T T T G A A A A C T A C G A T A A C T C T A A A A G
             1810              1820              1830              1840              1850              1860
```

FIG.1B.(CONTINUED)

```
   ASP  THR  SER  SER  ASN  TYR  LYS  ARG  THR  THR  TYR  GLY  SER  ASN  VAL  THR  LEU  GLY  PHE  PRO
T G A T A C A T C C T C T A A C T A T A A G C G T A C G A C T T A C G G A A G T A A T G T T A C T T T A G G T T T C C C
           1870              1880              1890              1900              1910              1920

   VAL  ASN  GLU  ASN  ASN  SER  TYR  TYR  VAL  GLY  LEU  GLY  HIS  THR  TYR  ASN  LYS  ILE  SER  ASN
T G T A A A T G A A A A T A A C T C C T A T T A T G T A G G A T T A G G T C A T A C C T A T A A T A A A A T T A G T A A
           1930              1940              1950              1960              1970              1980

   PHE  ALA  LEU  GLU  TYR  ASN  ARG  ASN  LEU  TYR  ILE  GLN  SER  MET  LYS  PHE  LYS  GLY  ASN  GLY
C T T T G C T C T A G A A T A T A A C C G T A A T T T A T A T A T T C A A T C A A T G A A A T T T A A A G G T A A T G G
           1990              2000              2010              2020              2030              2040

   ILE  LYS  THR  ASN  ASP  PHE  ASP  PHE  SER  PHE  GLY  TRP  ASN  TYR  ASN  SER  LEU  ASN  ARG  GLY
C A T T A A A A C A A A T G A C T T T G A T T T T T C T T T T G G T T G G A A C T A T A A C A G C C T T A A T A G A G G
           2050              2060              2070              2080              2090              2100

   TYR  PHE  PRO  THR  LYS  GLY  VAL  LYS  ALA  SER  LEU  GLY  GLY  ARG  VAL  THR  ILE  PRO  GLY  SER
C T A T T T C C C A A C T A A A G G G G T T A A A G C A A G T C T T G G T G G A C G A G T T A C T A T T C C A G G T T C
           2110              2120              2130              2140              2150              2160

   ASP  ASN  LYS  TYR  TYR  LYS  LEU  SER  ALA  ASP  VAL  GLN  GLY  PHE  TYR  PRO  LEU  ASP  ARG  ASP
T G A T A A C A A A T A C T A C A A A C T A A G T G C A G A T G T A C A G G G T T T C T A C C C A T T A G A C A G A G A
           2170              2180              2190              2200              2210              2220

   HIS  LEU  TRP  VAL  VAL  SER  ALA  LYS  ALA  SER  ALA  GLY  TYR  ALA  ASN  GLY  PHE  GLY  ASN  LYS
T C A C C T C T G G G T T G T A T C T G C A A A A G C A T C T G C A G G A T A T G C A A A T G G T T T T G G A A A C A A
           2230              2240              2250              2260              2270              2280
```

FIG.1B.(CONTINUED)

ARG LEU PRO PHE TYR GLN THR TYR THR ALA GLY GLY ILE GLY SER LEU ARG GLY PHE ALA
GCGTTTACCGTTCTATCAAACTTATACAGCGGGTGGCATCGGTTCATTACGTGGTTTTGC
2290 2300 2310 2320 2330 2340

TYR GLY SER ILE GLY PRO ASN ALA ILE TYR ALA GLU TYR GLY ASN GLY SER GLY THR GLY
TTATGGTAGTATTGGACCTAACGCAATTTATGCCGAATATGGTAATGGTAGTGGTACTGG
2350 2360 2370 2380 2390 2400

THR PHE LYS LYS ILE SER SER ASP VAL ILE GLY GLY ASN ALA ILE ALA THR ALA SER ALA
TACTTTTAAGAAGATAAGTTCTGATGTGATTGGTGGTAATGCAATCGCTACAGCTAGCGC
2410 2420 2430 2440 2450 2460

GLU LEU ILE VAL PRO THR PRO PHE VAL SER ASP LYS SER GLN ASN THR VAL ARG THR SER
AGAGTTAATTGTGCCAACTCCATTTGTGAGCGATAAGAGCCAAAATACGGTCCGAACCTC
2470 2480 2490 2500 2510 2520

LEU PHE VAL ASP ALA ALA SER VAL TRP ASN THR LYS TRP LYS SER ASP LYS ASN GLY LEU
CTTATTTGTTGATGCGGCAAGTGTTTGGAATACTAAATGGAAATCAGATAAAAATGGATT
2530 2540 2550 2560 2570 2580

GLU SER ASP VAL LEU LYS ARG LEU PRO ASP TYR GLY LYS SER SER ARG ILE ARG ALA SER
AGAGAGCGATGTATTAAAAAGATTGCCTGATTATGGCAAATCAAGCCGTATTCGCGCCTC
2590 2600 2610 2620 2630 2640

FIG.1B.(CONTINUED)

```
   THR  GLY  VAL  GLY  PHE  GLN  TRP  GLN  SER  PRO  ILE  GLY  PRO  LEU  VAL  PHE  SER  TYR  ALA  LYS
T A C A G G T G T C G G A T T C C A A T G G C A A T C T C C T A T T G G G C C A T T G G T A T T C T C T T A T G C C A A
           2650           2660           2670           2680           2690           2700

   PRO  ILE  LYS  LYS  TYR  GLU  ASN  ASP  ASP  VAL  GLU  GLN  PHE  GLN  PHE  SER  ILE  GLY  GLY  SER
A C C A A T T A A A A A A T A T G A A A A T G A T G A T G T C G A A C A G T T C C A A T T T A G T A T T G G A G G T T C
           2710           2720           2730           2740           2750           2760

   PHE  ***  ***
T T T C T A A T A A A T T G A A C T T T T T T C T T C A T C A G A A C T C A A A A A C A A C G T T C T C T G C C T A A T
           2770           2780           2790           2800           2810           2820

T T A A T T G G G C A G A G A A A A T A T T A A A C C C A T C A T T T A A T T A A G G A T A T T T A T C A A A T G A A A
           2830           2840           2850           2860           2870           2880

A A C A T C G C A A A A G T A A C C G C A C T T G C T T T A G G T A T T G C A C T T G C T T C A G G C T A T G C T T C C
           2890           2900           2910           2920           2930           2940

G C T G A A G A A A A A A T T G C T T T C A T T A A T G C A C T T A T A T T T T T C A A
           2950           2960           2970           2980
```

FIG.1C.

DS-691-1-5 DNA, Minn A D15 sequence
IS THE SEQUENCE BEING TRANSLATED

```
AATCACTTACTGGCGATTTGTCATTAAATAATTTAAGTGGGCCAATTTCTATTGCAAAAG
          10        20        30        40        50        60

GTGCTGGCACATCAGCAAATATTGGATTGGTGTATTTTTTAAGTTTTATGGCACTGATTA
          70        80        90       100       110       120

GTGTAAATTTAGGGATTATGAATTTATTTCCATTACCAGTATTAGATGGCGGTCATTTAG
         130       140       150       160       170       180

TTTTTTTAACAATGGAAGCTGTTAAAGGAAAACCTGTTTCTGAGCGGGTGCAAAGCATCT
         190       200       210       220       230       240

GTTATCGAATTGGCGCAGCACTGTTATTAAGCTTAACGGTGTTTGCATTATTTAATGATT
         250       260       270       280       290       300
```

FIG.1C.(CONTINUED)

```
                                                                MET  LYS  LYS  LEU  LEU  ILE  ALA  SER  LEU
TTTTACGTCTATAATTTATATAGGATACAATCGATGAAAAAACTTCTAATCGCAAGTTTA
            310                 320                 330                 340                 350                 360

 LEU  PHE  GLY  THR  THR  THR  THR  VAL  PHE  ALA  ALA  PRO  PHE  VAL  ALA  LYS  ASP  ILE  ARG  VAL
TTATTCGGTACGACAACGACTGTGTTTGCCGCACCTTTTGTGGCAAAAGATATTCGTGTG
            370                 380                 390                 400                 410                 420

 ASP  GLY  VAL  GLN  GLY  ASP  LEU  GLU  GLN  GLN  ILE  ARG  ALA  SER  LEU  PRO  VAL  ARG  ALA  GLY
GATGGTGTTCAAGGTGACTTAGAACAACAAATCCGAGCAAGTTTACCTGTTCGTGCCGGT
            430                 440                 450                 460                 470                 480

 GLN  ARG  VAL  THR  ASP  ASN  ASP  VAL  ALA  ASN  ILE  VAL  ARG  SER  LEU  PHE  VAL  SER  GLY  ARG
CAGCGTGTGACTGACAATGATGTGGCTAATATTGTCCGCTCTTTATTCGTAAGTGGTCGA
            490                 500                 510                 520                 530                 540

 PHE  ASP  ASP  VAL  LYS  ALA  HIS  GLN  GLU  GLY  ASP  VAL  LEU  VAL  VAL  SER  VAL  VAL  ALA  LYS
TTCGATGATGTGAAAGCGCATCAAGAAGGCGATGTGCTTGTTGTTAGCGTTGTGGCTAAA
            550                 560                 570                 580                 590                 600

 SER  ILE  ILE  SER  ASP  VAL  LYS  ILE  LYS  GLY  ASN  SER  VAL  ILE  PRO  THR  GLU  ALA  LEU  LYS
TCGATCATTTCAGATGTTAAAATCAAAGGTAACTCTGTTATTCCCACTGAAGCACTTAAA
            610                 620                 630                 640                 650                 660
```

FIG.1C.(CONTINUED)

```
 GLN  ASN  LEU  ASP  ALA  ASN  GLY  PHE  LYS  VAL  GLY  ASP  VAL  LEU  ILE  ARG  GLU  LYS  LEU  ASN
CAAAACTTAGATGCTAACGGGTTTAAAGTTGGCGATGTTTTAATTCGAGAAAAATTAAAT
          670            680            690            700            710            720

 GLU  PHE  ALA  LYS  SER  VAL  LYS  GLU  HIS  TYR  ALA  SER  VAL  GLY  ARG  TYR  ASN  ALA  THR  VAL
GAATTTGCCAAAAGTGTAAAAGAGCACTATGCAAGTGTAGGTCGCTATAACGCAACAGTT
          730            740            750            760            770            780

 GLU  PRO  ILE  VAL  ASN  THR  LEU  PRO  ASN  ASN  ARG  ALA  GLU  ILE  LEU  ILE  GLN  ILE  ASN  GLU
GAACCTATTGTCAATACGCTACCAAATAATCGCGCTGAAATTTTAATTCAAATCAATGAA
          790            800            810            820            830            840

 ASP  ASP  LYS  ALA  LYS  LEU  ALA  SER  LEU  THR  PHE  LYS  GLY  ASN  GLU  SER  VAL  SER  SER  SER
GATGATAAAGCAAAATTGGCATCATTAACTTTCAAGGGGAACGAATCTGTTAGTAGCAGT
          850            860            870            880            890            900

 THR  LEU  GLN  GLU  GLN  MET  GLU  LEU  GLN  PRO  ASP  SER  TRP  TRP  LYS  LEU  TRP  GLY  ASN  LYS
ACATTACAAGAACAAATGGAATTACAACCTGATTCTTGGTGGAAATTATGGGGAAATAAA
          910            920            930            940            950            960

 PHE  GLU  GLY  ALA  GLN  PHE  GLU  LYS  ASP  LEU  GLN  SER  ILE  ARG  ASP  TYR  TYR  LEU  ASN  ASN
TTTGAAGGTGCGCAATTCGAGAAAGATTTGCAGTCAATTCGTGATTATTATTTAAATAAT
          970            980            990           1000           1010           1020
```

FIG.1C.(CONTINUED)

GLY TYR ALA LYS ALA GLN ILE THR LYS THR ASP VAL GLN LEU ASN ASP GLU LYS THR LYS
GGCTATGCCAAAGCACAAATTACTAAAACGGATGTTCAGCTAAATGATGAAAAAACAAAA
1030 1040 1050 1060 1070 1080

VAL ASN VAL THR ILE ASP VAL ASN GLU GLY LEU GLN TYR ASP LEU ARG SER ALA ARG ILE
GTTAATGTAACCATTGATGTAAATGAAGGTTTACAGTATGACCTTCGTAGTGCACGCATT
1090 1100 1110 1120 1130 1140

ILE GLY ASN LEU GLY GLY MET SER ALA GLU LEU GLU PRO LEU LEU SER ALA LEU HIS LEU
ATAGGTAATCTGGGAGGTATGTCTGCCGAGCTTGAACCTTTACTTTCAGCATTACATTTA
1150 1160 1170 1180 1190 1200

ASN ASP THR PHE ARG ARG SER ASP ILE ALA ASP VAL GLU ASN ALA ILE LYS ALA LYS LEU
AATGATACTTTCCGCCGTAGTGATATTGCAGATGTAGAAAATGCAATTAAAGCAAAACTT
1210 1220 1230 1240 1250 1260

GLY GLU ARG GLY TYR GLY SER ALA THR VAL ASN SER VAL PRO ASP PHE ASP ASP ALA ASN
GGAGAACGCGGTTACGGTAGCGCAACGGTAAATTCAGTACCTGATTTTGATGATGCAAAT
1270 1280 1290 1300 1310 1320

LYS THR LEU ALA ILE THR LEU VAL VAL ASP ALA GLY ARG ARG LEU THR VAL ARG GLN LEU
AAAACATTAGCGATAACCCTTGTTGTTGATGCTGGACGACGTTTAACTGTTCGCCAACTT
1330 1340 1350 1360 1370 1380

FIG.1C.(CONTINUED)

```
 ARG  PHE  GLU  GLY  ASN  THR  VAL  SER  ALA  ASP  SER  THR  LEU  ARG  GLN  GLU  MET  ARG  GLN  GLN
C G C T T T G A A G G A A A T A C C G T T T C T G C T G A T A G C A C T T T A C G T C A G G A A A T G C G C C A A C A A
          1390              1400              1410              1420              1430              1440

 GLU  GLY  THR  TRP  TYR  ASN  SER  GLN  LEU  VAL  GLU  LEU  GLY  LYS  ILE  ARG  LEU  ASP  ARG  THR
G A A G G A A C T T G G T A T A A T T C A C A A T T A G T T G A G T T A G G A A A A A T T C G C T T A G A T C G T A C A
          1450              1460              1470              1480              1490              1500

 GLY  PHE  PHE  GLU  THR  VAL  GLU  ASN  ARG  ILE  ASP  PRO  ILE  ASN  GLY  SER  ASN  ASP  GLU  VAL
G G T T T C T T C G A A A C A G T C G A A A A C C G A A T T G A T C C T A T C A A T G G T A G T A A T G A T G A A G T G
          1510              1520              1530              1540              1550              1560

 ASP  VAL  VAL  TYR  LYS  VAL  LYS  GLU  ARG  ASN  THR  GLY  SER  ILE  ASN  PHE  GLY  ILE  GLY  TYR
G A T G T C G T A T A T A A A G T C A A A G A A C G T A A C A C G G G T A G T A T C A A C T T T G G T A T T G G T T A C
          1570              1580              1590              1600              1610              1620

 GLY  THR  GLU  SER  GLY  ILE  SER  TYR  GLN  ALA  SER  VAL  LYS  GLN  ASP  ASN  PHE  LEU  GLY  THR
G G T A C A G A G A G T G G T A T T A G T T A T C A A G C A A G T G T T A A A C A A G A T A A T T T C T T G G G A A C A
          1630              1640              1650              1660              1670              1680

 GLY  ALA  ALA  VAL  SER  ILE  ALA  GLY  THR  LYS  ASN  ASP  TYR  GLY  THR  SER  VAL  ASN  LEU  GLY
G G G G C G G C A G T A A G T A T A G C T G G T A C G A A A A A T G A T T A T G G T A C G A G T G T C A A T T T G G G T
          1690              1700              1710              1720              1730              1740
```

FIG.1C.(CONTINUED)

```
 TYR  THR  GLU  PRO  TYR  PHE  THR  LYS  ASP  GLY  VAL  SER  LEU  GLY  GLY  ASN  VAL  PHE  PHE  GLU
T A T A C C G A G C C C T A T T T T A C T A A A G A T G G T G T A A G T C T T G G T G G A A A T G T T T T C T T T G A A
          1750              1760              1770              1780              1790              1800

 ASN  TYR  ASP  ASN  SER  LYS  SER  ASP  THR  SER  SER  ASN  TYR  LYS  ARG  THR  THR  TYR  GLY  SER
A A C T A C G A T A A C T C T A A A A G T G A T A C A T C C T C T A A C T A T A A G C G T A C G A C T T A C G G A A G T
          1810              1820              1830              1840              1850              1860

 ASN  VAL  THR  LEU  GLY  PHE  PRO  VAL  ASN  GLU  ASN  ASN  SER  TYR  TYR  VAL  GLY  LEU  GLY  HIS
A A T G T T A C T T T A G G T T T C C C T G T A A A T G A A A A T A A C T C C T A T T A T G T A G G A T T A G G T C A T
          1870              1880              1890              1900              1910              1920

 THR  TYR  ASN  LYS  ILE  SER  ASN  PHE  ALA  LEU  GLU  TYR  ASN  ARG  ASN  LEU  TYR  ILE  GLN  SER
A C C T A T A A T A A A A T T A G T A A C T T T G C T C T A G A A T A T A A C C G T A A T T T A T A T A T T C A A T C A
          1930              1940              1950              1960              1970              1980

 MET  LYS  PHE  LYS  GLY  ASN  GLY  ILE  LYS  THR  ASN  ASP  PHE  ASP  PHE  SER  PHE  GLY  TRP  ASN
A T G A A A T T T A A A G G T A A T G G C A T T A A A A C A A A T G A C T T T G A T T T T T C T T T T G G T T G G A A C
          1990              2000              2010              2020              2030              2040

 TYR  ASN  SER  LEU  ASN  ARG  GLY  TYR  PHE  PRO  THR  LYS  GLY  VAL  LYS  ALA  SER  LEU  GLY  GLY
T A T A A C A G C C T T A A T A G A G G C T A T T T C C C A A C T A A A G G G G T T A A A G C A A G T C T T G G T G G A
          2050              2060              2070              2080              2090              2100
```

FIG.1C.(CONTINUED)

```
 ARG  VAL  THR  ILE  PRO  GLY  SER  ASP  ASN  LYS  TYR  TYR  LYS  LEU  SER  ALA  ASP  VAL  GLN  GLY
CGAGTTACTATTCCAGGTTCTGATAACAAATACTACAAACTAAGTGCAGATGTACAGGGT
          2110          2120          2130          2140          2150          2160

 PHE  TYR  PRO  LEU  ASP  ARG  ASP  HIS  LEU  TRP  VAL  VAL  SER  ALA  LYS  ALA  SER  ALA  GLY  TYR
TTCTACCCATTAGACAGAGATCACCTCTGGGTTGTATCTGCAAAAGCATCTGCAGGATAT
          2170          2180          2190          2200          2210          2220

 ALA  ASN  GLY  PHE  GLY  ASN  LYS  ARG  LEU  PRO  PHE  TYR  GLN  THR  TYR  THR  ALA  GLY  GLY  ILE
GCAAATGGTTTTGGAAACAAGCGTTTACCGTTCTATCAAACTTATACAGCGGGTGGCATC
          2230          2240          2250          2260          2270          2280

 GLY  SER  LEU  ARG  GLY  PHE  ALA  TYR  GLY  SER  ILE  GLY  PRO  ASN  ALA  ILE  TYR  ALA  GLU  TYR
GGTTCATTACGTGGTTTTGCTTATGGTAGTATTGGACCTAACGCAATTTATGCCGAATAT
          2290          2300          2310          2320          2330          2340

 GLY  ASN  GLY  SER  GLY  THR  GLY  THR  PHE  LYS  LYS  ILE  SER  SER  ASP  VAL  ILE  GLY  GLY  ASN
GGTAATGGTAGTGGTACTGGTACTTTTAAGAAGATAAGTTCTGATGTGATTGGTGGTAAT
          2350          2360          2370          2380          2390          2400

 ALA  ILE  ALA  THR  ALA  SER  ALA  GLU  LEU  ILE  VAL  PRO  THR  PRO  PHE  VAL  SER  ASP  LYS  SER
GCAATCGCTACAGCTAGCGCAGAGTTAATTGTGCCAACTCCATTTGTGAGCGATAAGAGC
          2410          2420          2430          2440          2450          2460
```

FIG.1C.(CONTINUED)

```
 GLN  ASN  THR  VAL  ARG  THR  SER  LEU  PHE  VAL  ASP  ALA  ALA  SER  VAL  TRP  ASN  THR  LYS  TRP
C A A A A T A C G G T C C G A A C C T C C T T A T T T G T T G A T G C G G C A A G T G T T T G G A A T A C T A A A T G G
          2470           2480           2490           2500           2510           2520

 LYS  SER  ASP  LYS  ASN  GLY  LEU  GLU  SER  ASP  VAL  LEU  LYS  ARG  LEU  PRO  ASP  TYR  GLY  LYS
A A A T C A G A T A A A A A T G G A T T A G A G A G C G A T G T A T T A A A A A G A T T G C C T G A T T A T G G C A A A
          2530           2540           2550           2560           2570           2580

 SER  SER  ARG  ILE  ARG  ALA  SER  THR  GLY  VAL  GLY  PHE  GLN  TRP  GLN  SER  PRO  ILE  GLY  PRO
T C A A G C C G T A T T C G C G C C T C T A C A G G T G T C G G A T T C C A A T G G C A A T C T C C T A T T G G G C C A
          2590           2600           2610           2620           2630           2640

 LEU  VAL  PHE  SER  TYR  ALA  LYS  PRO  ILE  LYS  LYS  TYR  GLU  ASN  ASP  ASP  VAL  GLU  GLN  PHE
T T G G T A T T C T C T T A T G C C A A A C C A A T T A A A A A A T A T G A A A A T G A T G A T G T C G A A C A G T T C
          2650           2660           2670           2680           2690           2700

 GLN  PHE  SER  ILE  GLY  GLY  SER  PHE  ***  ***
C A A T T T A G T A T T G G A G G T T C T T T C T A A T A A A T T G A A C T T T T T T C T T C A T C A G A A C T C A A A
          2710           2720           2730           2740           2750           2760
```

FIG.1C.(CONTINUED)

```
AACAACGTTCTCTGCCTAATTTAATTGGGCAGAGAAAATATTAAACCCATCATTTAATTA
          2770      2780      2790      2800      2810      2820

AGGATATTTATCAAATGAAAAACATCGCAAAAGTAACCGCACTTGCTTTAGGTATTGCAC
          2830      2840      2850      2860      2870      2880

TTGCTTCAGGCTATGCTTCCGCTGAAGAAAAAATTGCTTTCATTAATGCGGGTTATANTT
          2890      2900      2910      2920      2930      2940

TNCAAGGCNAAGG
          2950
```

FIG.1D.

SB33 D15

IS THE SEQUENCE BEING TRANSLATED

```
GGCATTGAAAAAACAGGACAGCTTTCCCTTTTAACCTTGAAAATATTAGGGAAATTACTT
          10        20        30        40        50        60

ACTGGCGATTTGTCATTAAATAATTTAAGTGGGCCAATTTCTATTGCAAAAGGTGCTGGC
          70        80        90       100       110       120

GCATCAGCAAATATTGGATTGGTGTATTTTTTAAGTTTTATGGCATTGATTAGTGTAAAT
         130       140       150       160       170       180

TTAGGGATTATGAATTTATTTCCATTACCAGTATTAGATGGCGGTCATTTAGTTTTTTTA
         190       200       210       220       230       240

ACAATGGAAGCTGTTAAAGGAAAACCTGTTTCTGAGCGGGTGCAAAGCATCTGTTATCGA
         250       260       270       280       290       300
```

FIG.1D.(CONTINUED)

```
ATTGGCGCAGCACTGTTATTAAGCTTAACGGTGTTTGCATTATTTAATGATTTTTTACGT
          310        320        330        340        350        360

                                  MET  LYS  LYS  LEU  LEU  ILE  ALA  SER  LEU  LEU  PHE  GLY
CTATAATTTATATAGGATACAATCGATGAAAAAACTTCTAATCGCAAGTTTATTATTCGG
          370        380        390        400        410        420

  THR  THR  THR  THR  VAL  PHE  ALA  ALA  PRO  PHE  VAL  ALA  LYS  ASP  ILE  ARG  VAL  ASP  GLY  VAL
TACGACAACGACTGTGTTTGCCGCACCTTTTGTGGCAAAAGATATTCGTGTGGATGGTGT
          430        440        450        460        470        480

  GLN  GLY  ASP  LEU  GLU  GLN  GLN  ILE  ARG  ALA  SER  LEU  PRO  VAL  ARG  ALA  GLY  GLN  ARG  VAL
TCAAGGTGACTTAGAACAACAAATCCGAGCAAGTTTACCTGTTCGTGCCGGTCAGCGTGT
          490        500        510        520        530        540

  THR  ASP  ASN  ASP  VAL  ALA  ASN  ILE  VAL  ARG  SER  LEU  PHE  VAL  SER  GLY  ARG  PHE  ASP  ASP
GACTGACAATGATGTGGCTAATATTGTCCGCTCTTTATTCGTAAGTGGTCGATTCGATGA
          550        560        570        580        590        600

  VAL  LYS  ALA  HIS  GLN  GLU  GLY  ASP  VAL  LEU  VAL  VAL  SER  VAL  VAL  ALA  LYS  SER  ILE  ILE
TGTGAAAGCGCATCAAGAAGGCGATGTGCTTGTTGTTAGCGTTGTGGCTAAATCGATCAT
          610        620        630        640        650        660
```

FIG.1D.(CONTINUED)

```
   SER  ASP  VAL  LYS  ILE  LYS  GLY  ASN  SER  ILE  ILE  PRO  PRO  GLU  ALA  LEU  LYS  GLN  ASN  LEU
T T C A G A T G T T A A A A T C A A A G G T A A C T C T A T T A T T C C A C C T G A A G C A C T A A A A C A A A A C T T
                670                  680                  690                  700                  710                  720

   ASP  ALA  ASN  GLY  PHE  LYS  VAL  GLY  ASP  ILE  LEU  ILE  ARG  GLU  LYS  LEU  ASN  GLU  PHE  ALA
A G A T G C T A A C G G G T T T A A A G T T G G C G A T A T T T T A A T T C G A G A A A A A T T A A A T G A A T T T G C
                730                  740                  750                  760                  770                  780

   GLN  SER  VAL  LYS  GLU  HIS  TYR  ALA  SER  VAL  GLY  ARG  TYR  ASN  ALA  THR  VAL  GLU  PRO  ILE
C C A A A G T G T A A A A G A G C A C T A T G C A A G T G T A G G T C G C T A T A A C G C A A C C G T T G A A C C T A T
                790                  800                  810                  820                  830                  840

   VAL  ASN  THR  LEU  PRO  ASN  ASN  ARG  ALA  GLU  ILE  LEU  ILE  GLN  ILE  ASN  GLU  ASP  ASP  LYS
T G T C A A T A C G C T A C C A A A T A A T C G C G C T G A A A T T T T A A T T C A A A T C A A T G A A G A T G A T A A
                850                  860                  870                  880                  890                  900

   ALA  LYS  LEU  ALA  SER  LEU  THR  PHE  LYS  GLY  ASN  GLU  SER  VAL  SER  SER  SER  THR  LEU  GLN
A G C C A A A T T G G C A T C A T T A A C T T T C A A G G G G A A C G A A T C T G T T A G T A G C A G T A C A T T A C A
                910                  920                  930                  940                  950                  960

   GLU  GLN  MET  GLU  LEU  GLN  PRO  ASP  SER  TRP  TRP  LYS  LEU  TRP  GLY  ASN  LYS  PHE  GLU  GLY
A G A A C A A A T G G A A T T A C A A C C T G A T T C T T G G T G G A A A T T A T G G G G A A A T A A A T T T G A A G G
                970                  980                  990                  1000                 1010                 1020
```

FIG.1D.(CONTINUED)

```
   ALA  GLN  PHE  GLU  LYS  ASP  LEU  GLN  ALA  ILE  ARG  ASP  TYR  TYR  LEU  ASN  ASN  GLY  TYR  ALA
TGCGCAATTCGAGAAAGATTTGCAGGCAATTCGTGATTATTATTTAAATAATGGCTATGC
            1030          1040          1050          1060          1070          1080

   LYS  ALA  GLN  ILE  THR  LYS  ALA  ASP  VAL  GLN  LEU  ASN  ASP  GLU  LYS  THR  LYS  VAL  ASN  VAL
CAAAGCACAAATCACTAAAGCGGATGTTCAGCTAAATGATGAAAAAACAAAAGTTAATGT
            1090          1100          1110          1120          1130          1140

   THR  ILE  ASP  VAL  ASN  GLU  GLY  LEU  GLN  TYR  ASP  LEU  ARG  SER  ALA  ARG  ILE  ILE  GLY  ASN
AACCATTGATGTAAATGAAGGTTTACAGTATGACCTTCGTAGTGCACGCATTATAGGTAA
            1150          1160          1170          1180          1190          1200

   LEU  GLY  GLY  MET  SER  ALA  GLU  LEU  GLU  PRO  LEU  LEU  SER  ALA  LEU  HIS  LEU  ASN  ASP  THR
TCTGGGAGGTATGTCTGCCGAGCTTGAACCTTTACTTTCAGCATTACATTTAAATGATAC
            1210          1220          1230          1240          1250          1260

   PHE  ARG  ARG  SER  ASP  ILE  ALA  ASP  VAL  GLU  ASN  ALA  ILE  LYS  ALA  LYS  LEU  GLY  GLU  ARG
TTTCCGCCGTAGTGATATTGCAGATGTAGAAAATGCAATTAAAGCAAAACTTGGGGAACG
            1270          1280          1290          1300          1310          1320

   GLY  TYR  GLY  ASN  THR  THR  VAL  ASN  SER  VAL  PRO  ASP  PHE  ASP  ASP  ALA  ASN  LYS  THR  LEU
AGGTTACGGTAACACAACAGTAAATTCTGTACCTGATTTTGACGATGCAAATAAAACATT
            1330          1340          1350          1360          1370          1380
```

FIG.1D.(CONTINUED)

```
   ALA  ILE  THR  PHE  VAL  VAL  ASP  ALA  GLY  ARG  ARG  LEU  THR  VAL  HIS  GLN  LEU  ARG  PHE  GLU
AGCGATAACCTTTGTTGTTGATGCTGGACGACGTTTAACTGTTCACCAACTTCGCTTTGA
           1390          1400          1410          1420          1430          1440

   GLY  ASN  THR  VAL  SER  ALA  ASP  SER  THR  LEU  ARG  GLN  GLU  MET  ARG  GLN  GLN  GLU  GLY  THR
AGGAAATACCGTTTCTGCTGATAGTACTTTACGTCAGGAAATGCGCCAACAAGAAGGAAC
           1450          1460          1470          1480          1490          1500

   TRP  TYR  ASN  SER  GLN  LEU  VAL  GLU  LEU  GLY  LYS  ILE  ARG  LEU  ASP  ARG  THR  GLY  PHE  PHE
TTGGTATAATTCACAATTAGTTGAGTTAGGAAAAATTCGCTTAGATCGTACAGGTTTCTT
           1510          1520          1530          1540          1550          1560

   GLU  THR  VAL  GLU  ASN  ARG  ILE  ASP  PRO  ILE  ASN  GLY  SER  ASN  ASP  GLU  VAL  ASP  VAL  VAL
CGAAACAGTTGAAAACCGAATTGATCCTATCAATGGTAGCAATGATGAAGTGGATGTCGT
           1570          1580          1590          1600          1610          1620

   TYR  LYS  VAL  LYS  GLU  ARG  ASN  THR  GLY  SER  ILE  ASN  PHE  GLY  ILE  GLY  TYR  GLY  THR  GLU
ATATAAAGTCAAAGAACGTAACACGGGTAGTATCAACTTTGGTATTGGTTACGGTACAGA
           1630          1640          1650          1660          1670          1680

   SER  GLY  ILE  SER  TYR  GLN  ALA  SER  VAL  LYS  GLN  ASP  ASN  PHE  LEU  GLY  THR  GLY  ALA  ALA
GAGTGGTATTAGTTATCAAGCAAGTGTCAAACAAGATAATTTCTTGGGAACAGGGGCGGC
           1690          1700          1710          1720          1730          1740
```

FIG.1D.(CONTINUED)

```
  VAL  SER  ILE  ALA  GLY  THR  LYS  ASN  ASP  TYR  GLY  THR  SER  VAL  ASN  LEU  GLY  TYR  THR  GLU
AGTAAGTATAGCTGGTACGAAAAATGATTATGGTACGAGTGTCAATTTGGGTTATACCGA
             1750         1760         1770         1780         1790         1800

  PRO  TYR  PHE  THR  LYS  ASP  GLY  VAL  SER  LEU  GLY  GLY  ASN  VAL  PHE  PHE  GLU  ASN  TYR  ASP
GCCCTATTTTACTAAAGATGGTGTAAGTCTTGGTGGAAATGTTTTCTTTGAAAACTACGA
             1810         1820         1830         1840         1850         1860

  ASN  SER  LYS  SER  ASP  THR  SER  SER  ASN  TYR  LYS  ARG  THR  THR  TYR  GLY  SER  ASN  VAL  THR
TAACTCTAAAAGTGATACATCCTCTAACTATAAGCGTACGACTTATGGAAGTAATGTTAC
             1870         1880         1890         1900         1910         1920

  LEU  GLY  PHE  PRO  VAL  ASN  GLU  ASN  ASN  SER  TYR  TYR  VAL  GLY  LEU  GLY  HIS  THR  TYR  ASN
TTTAGGTTTCCCTGTAAATGAAAATAACTCCTATTATGTAGGATTAGGCCATACCTATAA
             1930         1940         1950         1960         1970         1980

  LYS  ILE  SER  ASN  PHE  ALA  LEU  GLU  TYR  ASN  ARG  ASN  LEU  TYR  ILE  GLN  SER  MET  LYS  PHE
TAAAATTAGTAACTTTGCTCTAGAATATAACCGTAATTTATATATTCAATCAATGAAATT
             1990         2000         2010         2020         2030         2040

  LYS  GLY  ASN  GLY  ILE  LYS  THR  ASN  ASP  PHE  ASP  PHE  SER  PHE  GLY  TRP  ASN  TYR  ASN  SER
TAAAGGTAATGGCATTAAAACAAATGACTTTGATTTTTCTTTTGGTTGGAACTATAACAG
             2050         2060         2070         2080         2090         2100
```

FIG.1D.(CONTINUED)

```
   LEU  ASN  ARG  GLY  TYR  PHE  PRO  THR  LYS  GLY  VAL  LYS  ALA  SER  LEU  GLY  GLY  ARG  VAL  THR
C C T T A A T A G A G G C T A T T T C C C A A C T A A A G G G G T T A A A G C A A G T C T T G G T G G A C G A G T T A C
             2110              2120              2130              2140              2150              2160

   ILE  PRO  GLY  SER  ASP  ASN  LYS  TYR  TYR  LYS  LEU  SER  ALA  ASP  VAL  GLN  GLY  PHE  TYR  PRO
A A T T C C A G G T T C T G A T A A C A A A T A C T A C A A A C T A A G T G C A G A T G T A C A G G G T T T C T A C C C
             2170              2180              2190              2200              2210              2220

   LEU  ASP  ARG  ASP  HIS  LEU  TRP  VAL  VAL  SER  ALA  LYS  ALA  SER  ALA  GLY  TYR  ALA  ASN  GLY
A T T A G A C A G A G A T C A C C T C T G G G T T G T A T C T G C A A A A G C A T C T G C A G G A T A T G C A A A T G G
             2230              2240              2250              2260              2270              2280

   PHE  GLY  ASN  LYS  ARG  LEU  PRO  PHE  TYR  GLN  THR  TYR  THR  ALA  GLY  GLY  ILE  GLY  SER  LEU
T T T T G G A A A C A A G C G T T T A C C G T T C T A T C A A A C T T A T A C A G C G G G T G G C A T T G G T T C A T T
             2290              2300              2310              2320              2330              2340

   ARG  GLY  PHE  ALA  TYR  GLY  SER  ILE  GLY  PRO  ASN  ALA  ILE  TYR  GLN  GLY  GLN  ASN  ASN  LYS
A C G C G G T T T T G C T T A T G G T A G C A T T G G G C C T A A C G C A A T T T A T C A A G G T C A A A A T A A T A A
             2350              2360              2370              2380              2390              2400

   PHE  ASN  LYS  ILE  SER  SER  ASP  VAL  ILE  GLY  GLY  ASN  ALA  ILE  ALA  THR  ALA  SER  ALA  GLU
A T T T A A T A A G A T A A G T T C T G A T G T G A T T G G T G G T A A T G C A A T C G C T A C A G C T A G C G C A G A
             2410              2420              2430              2440              2450              2460
```

FIG.1D.(CONTINUED)

```
   LEU  ILE  VAL  PRO  THR  PRO  PHE  VAL  SER  ASP  LYS  SER  GLN  ASN  THR  VAL  ARG  THR  SER  LEU
G T T A A T T G T G C C A A C T C C A T T T G T G A G T G A T A A G A G T C A A A A T A C A G T C C G A A C C T C C C T
            2470              2480              2490              2500              2510              2520

   PHE  VAL  ASP  ALA  ALA  SER  VAL  TRP  ASN  THR  LYS  TRP  LYS  SER  ASP  LYS  ASN  GLY  LEU  GLU
A T T T G T T G A T G C G G C A A G T G T T T G G A A T A C T A A A T G G A A A T C A G A T A A A A A T G G A T T A G A
            2530              2540              2550              2560              2570              2580

   SER  ASN  VAL  LEU  LYS  ASP  LEU  PRO  ASP  TYR  GLY  LYS  SER  SER  ARG  THR  ARG  ALA  SER  THR
G A G C A A T G T C T T G A A A G A C T T A C C C G A T T A T G G C A A A T C A A G C C G T A C T C G C G C C T C T A C
            2590              2600              2610              2620              2630              2640

   GLY  VAL  GLY  PHE  GLN  TRP  GLN  SER  PRO  SER  GLY  PRO  VAL  VAL  PHE  SER  TYR  ALA  LYS  PRO
A G G T G T C G G A T T C C A A T G G C A A T C T C C T A G T G G A C C A G T G G T A T T T T C T T A T G C T A A A C C
            2650              2660              2670              2680              2690              2700

   ILE  LYS  LYS  TYR  GLU  ASN  ASP  ASP  VAL  GLU  GLN  PHE  GLN  PHE  SER  ILE  GLY  GLY  SER  PHE
A A T T A A A A A A T A T G A A A A T G A T G A T G T C G A A C A G T T C C A A T T T A G T A T T G G G G G T T C T T T
            2710              2720              2730              2740              2750              2760

   ***  ***
C T A A T A A A T T G A A C T T T T T T C G T C A T C A G A A C T C A A A A A C A A C G T T C T C T G C C T A A T T T A
            2770              2780              2790              2800              2810              2820
```

FIG.1D.(CONTINUED)

```
ATTGGGCAGAGAAAATATTAAAACCATCATTTAATTAAGGATATTTATCAAATGAAAAAC
          2830          2840          2850          2860          2870          2880

ATCGCCAAAGTAACCGCACTTGCTTTAGGTATTGCACTTGCTTCAGGCTATGCTGCAGCT
          2890          2900          2910          2920          2930          2940

GAAGAAAAAATTGCTTTTATTAATGCAGGTTATA
          2950          2960          2970
```

FIG.1E.

JB-1042-9-4 DNA, PAK D15
IS THE SEQUENCE BEING TRANSLATED

```
AAAAGGCATTGAAAAAACAGGACAACTTTCCCTTTTAACCTTGAAAATATTAGGGAAATT
          10        20        30        40        50        60

ACTTACTGGCGATTTGTCATTAAATAATTTAAGTGGGCCAATTTCTATTGCAAAAGGTGC
          70        80        90       100       110       120

TGGTGCATCAGCAAATATTGGATTGGTGTATTTTTTAAGTTTTATGGCATTGATTAGTGT
         130       140       150       160       170       180

AAATTTAGGGATTATGAATTTATTTCCATTACCAGTATTAGATGGCGGTCATTTAGTTTT
         190       200       210       220       230       240

TTTAACAATGGAAGCTGTTAAAGGAAAACCTGTTTCTGAGCGGGTGCAAAGCATCTGTTA
         250       260       270       280       290       300

TCGAATTGGCGCAGCACTGTTATTAAGCTTAACGGTGTTTGCATTATTTAATGATTTTTT
         310       320       330       340       350       360
```

FIG.1E.(CONTINUED)

```
                                                                MET  LYS  LYS  LEU  LEU  ILE  ALA  SER  LEU  LEU  P
A C G T C T A T A A T T T A T A T A G G A T A C A A T C G A T G A A A A A A C T T C T A A T C G C A A G T T T A T T A T
                370                  380                  390                  400                  410                  420

HE  GLY  THR  THR  THR  THR  VAL  PHE  ALA  ALA  PRO  PHE  VAL  ALA  LYS  ASP  ILE  ARG  VAL  ASP  G
T C G G T A C G A C A A C G A C T G T G T T T G C C G C A C C T T T T G T G G C A A A A G A T A T T C G T G T G G A T G
                430                  440                  450                  460                  470                  480

LY  VAL  GLN  GLY  ASP  LEU  GLU  GLN  GLN  ILE  ARG  ALA  SER  LEU  PRO  VAL  ARG  ALA  GLY  GLN  A
G T G T T C A A G G T G A C T T A G A A C A A C A A A T C C G A G C A A G T T T A C C T G T T C G T G C T G G T C A G C
                490                  500                  510                  520                  530                  540

RG  VAL  THR  ASP  ASN  ASP  VAL  ALA  ASN  ILE  VAL  ARG  SER  LEU  PHE  VAL  SER  GLY  ARG  PHE  A
G T G T G A C T G A C A A T G A T G T G G C T A A T A T T G T C C G C T C T T T A T T C G T A A G T G G T C G A T T C G
                550                  560                  570                  580                  590                  600

SP  ASP  VAL  LYS  ALA  HIS  GLN  GLU  GLY  ASP  VAL  LEU  VAL  VAL  SER  VAL  VAL  ALA  LYS  SER  I
A T G A T G T G A A A G C G C A T C A A G A A G G C G A T G T G C T T G T T G T T A G C G T T G T G G C T A A A T C G A
                610                  620                  630                  640                  650                  660

LE  ILE  SER  ASP  VAL  LYS  ILE  LYS  GLY  ASN  SER  VAL  ILE  PRO  THR  GLU  ALA  LEU  LYS  GLN  A
T C A T T T C A G A T G T T A A A A T C A A A G G T A A C T C T G T T A T T C C C A C T G A A G C A C T T A A A C A A A
                670                  680                  690                  700                  710                  720
```

FIG.1E.(CONTINUED)

```
SN  LEU  ASP  ALA  ASN  GLY  PHE  LYS  VAL  GLY  ASP  VAL  LEU  ILE  ARG  GLU  LYS  LEU  ASN  GLU  P
A C T T A G A T G C T A A C G G G T T T A A A G T T G G C G A T G T T T T A A T T C G A G A A A A A T T A A A T G A A T
              730                  740                  750                  760                  770                  780

HE  ALA  LYS  SER  VAL  LYS  GLU  HIS  TYR  ALA  SER  VAL  GLY  ARG  TYR  ASN  ALA  THR  VAL  GLU  P
T T G C C A A A A G T G T A A A A G A G C A C T A T G C A A G T G T A G G T C G C T A T A A C G C A A C C G T T G A A C
              790                  800                  810                  820                  830                  840

RO  ILE  VAL  ASN  THR  LEU  PRO  ASN  ASN  ARG  ALA  GLU  ILE  LEU  ILE  GLN  ILE  ASN  GLU  ASP  A
C T A T T G T C A A T A C G C T G C C A A A T A A T C G T G C T G A A A T T T T A A T T C A A A T C A A T G A A G A T G
              850                  860                  870                  880                  890                  900

SP  LYS  ALA  LYS  LEU  ALA  SER  LEU  THR  PHE  LYS  GLY  ASN  GLU  SER  VAL  SER  SER  SER  THR  L
A T A A A G C A A A A T T G G C A T C A T T A A C T T T C A A G G G G A A C G A A T C T G T T A G T A G C A G T A C A T
              910                  920                  930                  940                  950                  960

EU  GLN  GLU  GLN  MET  GLU  LEU  GLN  PRO  ASP  SER  TRP  TRP  LYS  LEU  TRP  GLY  ASN  LYS  PHE  G
T A C A A G A A C A A A T G G A A T T A C A A C C T G A T T C T T G G T G G A A A T T A T G G G G A A A T A A A T T T G
              970                  980                  990                  1000                 1010                 1020

LU  GLY  ALA  GLN  PHE  GLU  LYS  ASP  LEU  GLN  ALA  ILE  ARG  ASP  TYR  TYR  LEU  ASN  ASN  GLY  T
A A G G T G C G C A A T T C G A G A A A G A T C T G C A G G C A A T T C G T G A T T A T T A T T T A A A T A A T G G C T
              1030                 1040                 1050                 1060                 1070                 1080

YR  ALA  LYS  ALA  GLN  ILE  THR  LYS  THR  ASP  VAL  GLN  LEU  ASN  ASP  GLU  LYS  THR  LYS  VAL  A
A T G C C A A A G C A C A A A T C A C T A A A A C G G A T G T T C A G C T A A A T G A T G A A A A A A C A A A A G T T A
              1090                 1100                 1110                 1120                 1130                 1140
```

FIG.1E.(CONTINUED)

```
SN  VAL  THR  ILE  ASP  VAL  ASN  GLU  GLY  LEU  GLN  TYR  ASP  LEU  ARG  SER  ALA  ARG  ILE  ILE  G
ATGTAACCATTGATGTAAATGAAGGTTTACAGTATGACCTTCGTAGTGCACGCATTATAG
          1150          1160          1170          1180          1190          1200

LY  ASN  LEU  GLY  GLY  MET  SER  ALA  GLU  LEU  GLU  PRO  LEU  LEU  SER  ALA  LEU  HIS  LEU  ASN  A
GTAATCTGGGAGGTATGTCTGCCGAGCTTGAACCTTTACTTTCAGCATTACATTTAAATG
          1210          1220          1230          1240          1250          1260

SP  THR  PHE  ARG  ARG  SER  ASP  ILE  ALA  ASP  VAL  GLU  ASN  ALA  ILE  LYS  ALA  LYS  LEU  GLY  G
ATACTTTCCGCCGTAGTGATATTGCAGATGTAGAAAATGCAATTAAAGCAAAACTTGGGG
          1270          1280          1290          1300          1310          1320

LU  ARG  GLY  TYR  GLY  ASN  THR  THR  VAL  ASN  SER  VAL  PRO  ASP  PHE  ASP  ASP  ALA  ASN  LYS  T
AACGAGGTTACGGTAACACAACAGTAAATTCTGTACCTGATTTTGACGATGCAAATAAAA
          1330          1340          1350          1360          1370          1380

HR  LEU  ALA  ILE  THR  PHE  VAL  VAL  ASP  ALA  GLY  ARG  ARG  LEU  THR  VAL  ARG  GLN  LEU  ARG  P
CATTAGCGATAACCTTTGTTGTTGATGCTGGACGACGTTTAACTGTTCGCCAACTTCGCT
          1390          1400          1410          1420          1430          1440

HE  GLU  GLY  ASN  THR  VAL  SER  ALA  ASP  SER  THR  LEU  ARG  GLN  GLU  MET  ARG  GLN  GLN  GLU  G
TTGAAGGAAATACCGTTTCTGCTGATAGTACTTTACGTCAGGAAATGCGACAACAAGAAG
          1450          1460          1470          1480          1490          1500
```

FIG.1E.(CONTINUED)

```
LY  THR  TRP  TYR  ASN  SER  GLN  LEU  VAL  GLU  LEU  GLY  LYS  ILE  ARG  LEU  ASP  ARG  THR  GLY  P
GAACTTGGTATAATTCACAATTAGTTGAGTTAGGAAAAATTCGCTTAGATCGTACAGGTT
           1510          1520          1530          1540          1550          1560

HE  PHE  GLU  THR  VAL  GLU  ASN  ARG  ILE  ASP  PRO  ILE  ASN  GLY  SER  ASN  ASP  GLU  VAL  ASP  V
TCTTCGAAACAGTTGAAAACCGAATTGATCCTATCAATGGTAGCAATGATGAAGTGGATG
           1570          1580          1590          1600          1610          1620

AL  VAL  TYR  LYS  VAL  LYS  GLU  ARG  ASN  THR  GLY  SER  ILE  ASN  PHE  GLY  ILE  GLY  TYR  GLY  T
TCGTATATAAAGTCAAAGAACGTAACACGGGTAGTATCAACTTTGGTATTGGTTACGGTA
           1630          1640          1650          1660          1670          1680

HR  GLU  SER  GLY  ILE  SER  TYR  GLN  THR  SER  ILE  LYS  GLN  ASP  ASN  PHE  LEU  GLY  THR  GLY  A
CAGAGAGTGGTATCAGTTATCAAACAAGTATTAAACAAGATAATTTCTTGGGAACAGGGG
           1690          1700          1710          1720          1730          1740

LA  ALA  VAL  SER  ILE  ALA  GLY  THR  LYS  ASN  ASP  TYR  GLY  THR  SER  VAL  ASN  LEU  GLY  TYR  T
CGGCAGTAAGTATAGCTGGTACGAAAAATGATTATGGTACGAGTGTCAATTTGGGTTATA
           1750          1760          1770          1780          1790          1800

HR  GLU  PRO  TYR  PHE  THR  LYS  ASP  GLY  VAL  SER  LEU  GLY  GLY  ASN  ILE  PHE  PHE  GLU  ASN  T
CCGAACCCTATTTTACTAAAGATGGTGTAAGTCTTGGTGGAAATATTTTCTTTGAAAACT
           1810          1820          1830          1840          1850          1860
```

FIG.1E.(CONTINUED)

YR ASP ASN SER LYS SER ASP THR SER SER ASN TYR LYS ARG THR THR TYR GLY SER ASN V
A C G A T A A C T C T A A A A G T G A T A C A T C C T C T A A C T A T A A G C G T A C G A C T T A T G G A A G T A A T G
1870 1880 1890 1900 1910 1920

AL THR LEU GLY PHE PRO VAL ASN GLU ASN ASN SER TYR TYR VAL GLY LEU GLY HIS THR T
T T A C T T T A G G T T T C C C T G T A A A T G A A A A T A A C T C C T A T T A T G T A G G A T T A G G C C A T A C C T
1930 1940 1950 1960 1970 1980

YR ASN LYS ILE SER ASN PHE ALA LEU GLU TYR ASN ARG ASN LEU TYR ILE GLN SER MET L
A T A A T A A A A T T A G T A A C T T T G C T C T A G A A T A T A A C C G T A A T T T A T A T A T T C A A T C A A T G A
1990 2000 2010 2020 2030 2040

YS PHE LYS GLY ASN GLY ILE LYS THR ASN ASP PHE ASP PHE SER PHE GLY TRP ASN TYR A
A A T T T A A A G G T A A T G G C A T T A A A A C A A A T G A C T T T G A T T T T T C T T T T G G T T G G A A C T A T A
2050 2060 2070 2080 2090 2100

SN SER LEU ASN ARG GLY TYR PHE PRO THR LYS GLY VAL LYS ALA SER LEU GLY GLY ARG V
A C A G C C T T A A T A G A G G C T A T T T C C C A A C T A A A G G G G T T A A A G C A A G T C T T G G T G G A C G A G
2110 2120 2130 2140 2150 2160

AL THR ILE PRO GLY SER ASP ASN LYS TYR TYR LYS LEU SER ALA ASP VAL GLN GLY PHE T
T T A C T A T T C C A G G T T C T G A T A A C A A A T A C T A C A A A C T A A G T G C A G A T G T A C A G G G T T T C T
2170 2180 2190 2200 2210 2220

YR PRO LEU ASP ARG ASP HIS ARG TRP VAL VAL SER ALA LYS ALA SER ALA GLY TYR ALA A
A C C C A T T A G A C A G A G A T C A C C G C T G G G T T G T A T C T G C A A A A G C A T C T G C A G G A T A T G C A A
2230 2240 2250 2260 2270 2280

FIG.1E.(CONTINUED)

```
SN  GLY  PHE  GLY  ASN  LYS  ARG  LEU  PRO  PHE  TYR  GLN  THR  TYR  THR  ALA  GLY  GLY  ILE  GLY  S
ATGGTTTTGGAAACAAGCGTTTACCGTTCTATCAAACTTATACAGCGGGTGGCATTGGTT
            2290         2300         2310         2320         2330         2340

ER  LEU  ARG  GLY  PHE  ALA  TYR  GLY  SER  ILE  GLY  PRO  ASN  ALA  ILE  TYR  ALA  GLU  HIS  GLY  A
CATTACGCGGTTTTGCTTATGGTAGTATTGGGCCTAATGCAATTTATGCCGAACATGGTA
            2350         2360         2370         2380         2390         2400

SN  GLY  THR  PHE  ASN  LYS  ILE  SER  SER  ASP  VAL  ILE  GLY  GLY  ASN  ALA  ILE  THR  THR  ALA  S
ATGGTACTTTTAATAAGATAAGTTCTGATGTGATTGGTGGTAATGCAATCACAACTGCGA
            2410         2420         2430         2440         2450         2460

ER  ALA  GLU  LEU  ILE  VAL  PRO  THR  PRO  PHE  VAL  SER  ASP  LYS  SER  GLN  ASN  THR  VAL  ARG  T
GTGCAGAACTTATTGTACCAACTCCATTTGTGAGTGATAAAAGCCAAAAATACAGTCCGAA
            2470         2480         2490         2500         2510         2520

HR  SER  LEU  PHE  VAL  ASP  ALA  ALA  SER  VAL  TRP  ASN  THR  LYS  TRP  LYS  SER  ASP  LYS  ASN  G
CCTCCCTATTTGTTGATGCGGCAAGTGTTTGGAATACTAAATGGAAATCAGATAAAAATG
            2530         2540         2550         2560         2570         2580

LY  LEU  GLU  SER  LYS  VAL  LEU  LYS  ASP  LEU  PRO  ASP  TYR  GLY  LYS  SER  SER  ARG  ILE  ARG  A
GATTAGAGAGCAAGGTCTTGAAAGACTTACCTGATTATGGCAAATCAAGCCGTATTCGCG
            2590         2600         2610         2620         2630         2640
```

FIG.1E.(CONTINUED)

```
LA  SER THR GLY VAL GLY PHE GLN TRP GLN SER PRO ILE GLY PRO LEU VAL PHE SER TYR A
CCTCTACAGGTGTCGGATTCCAATGGCAATCTCCTATTGGACCATTGGTATTTTCTTATG
          2650          2660          2670          2680          2690          2700

LA LYS PRO ILE LYS LYS TYR GLU ASN ASP ASP VAL GLU GLN PHE GLN PHE SER ILE GLY G
CTAAACCAATTAAAAAATATGAAAATGATGATGTCGAACAGTTCCAATTTAGTATTGGGG
          2710          2720          2730          2740          2750          2760

LY  SER PHE  ***  ***
GCTCTTTCTAATAAATTGAACTTTTTTCGTCATCAGAACTCAAAAACGACGTTCTCTGCC
          2770          2780          2790          2800          2810          2820

TAATTGAATTGGGCAGAGAAAATATTAAACCCATCATTTAATTAAGGATATTTATCAAAT
          2830          2840          2850          2860          2870          2880

GAAAAACATCGCAAAAGTAACCGCACTTGCTTTAGGTTTTGCACTTGCTTCAGGCTATGC
          2890          2900          2910          2920          2930          2940

TTCCGCTGAAGAAAAAATTGCTTTCATTAATGCAGGTTATATTTTTCAA
          2950          2960          2970          2980
```

FIG.1F.

1. cad15 (1-2949)
3. minnad15 (1-2953)
2. eagand15 (1-2984)
4. pakd15 (1-2989)
5. sb33d15 (1-2974)

```
cad15      1
minnad15   1
eagand15   1                    ACAGGACAgCTTTCCCTTTTAACCTTGAAAATATTAGGGAAATTA
                                |||||||| ||||||||||||||||||||||||||||||||||||
pakd15     1 aaaaGGCATTGAAAAAACAGGACAaCTTTCCCTTTTAACCTTGAAAATATTAGGGAAATTA
             |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
sb33d15    1     GGCATTGAAAAAACAGGACAgCTTTCCCTTTTAACCTTGAAAATATTAGGGAAATTA

consensus        aaaaggcattgaaaaaacaggacagctttccctttaaccttgaaaatattagggaaatta

cad15      1
```

FIG.1F.(CONTINUED)

```
minnad15      6 CTTaCTGGCGATTTGTCATTAAATAATTTAAGTGGGCCAATTTCTATTGCAAAAGGTGCTG
                |||  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15     46 CTTcCTGGCGATTTGTCATTAAATAATTTAAGTGGGCCAATTTCTATTGCAAAAGGTGCTG
                |||  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
pakd15       62 CTTACTGGCGATTTGTCATTAAATAATTTAAGTGGGCCAATTTCTATTGCAAAAGGTGCTG
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
sb33d15      58 CTTACTGGCGATTTGTCATTAAATAATTTAAGTGGGCCAATTTCTATTGCAAAAGGTGCTG

consensus       cttactggcgatttgtcattaaataatttaagtgggccaatttctattgcaaaaggtgctg

cad15         1

minnad15     67 GCaCATCAGCAAATATTGGATTGGTGTATTTTTTAAGTTTTATGGCACTGATTAGTGTAAA
                ||  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15    107 GCcCATCAGCAAATATTGGATTGGTGTATTTTTTAAGTTTTATGGCACTGATTAGTGTAAA
                |   |||||||||||||||||||||||||||||||||||||||||||  ||||||||||||
pakd15      123 GtGCATCAGCAAATATTGGATTGGTGTATTTTTTAAGTTTTATGGCATTGATTAGTGTAAA
                |  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
sb33d15     119 GcGCATCAGCAAATATTGGATTGGTGTATTTTTTAAGTTTTATGGCATTGATTAGTGTAAA

consensus       gcgcatcagcaaatattggattggtgtatttttaagttttatggca-tgattagtgtaaa
```

FIG.1F.(CONTINUED)

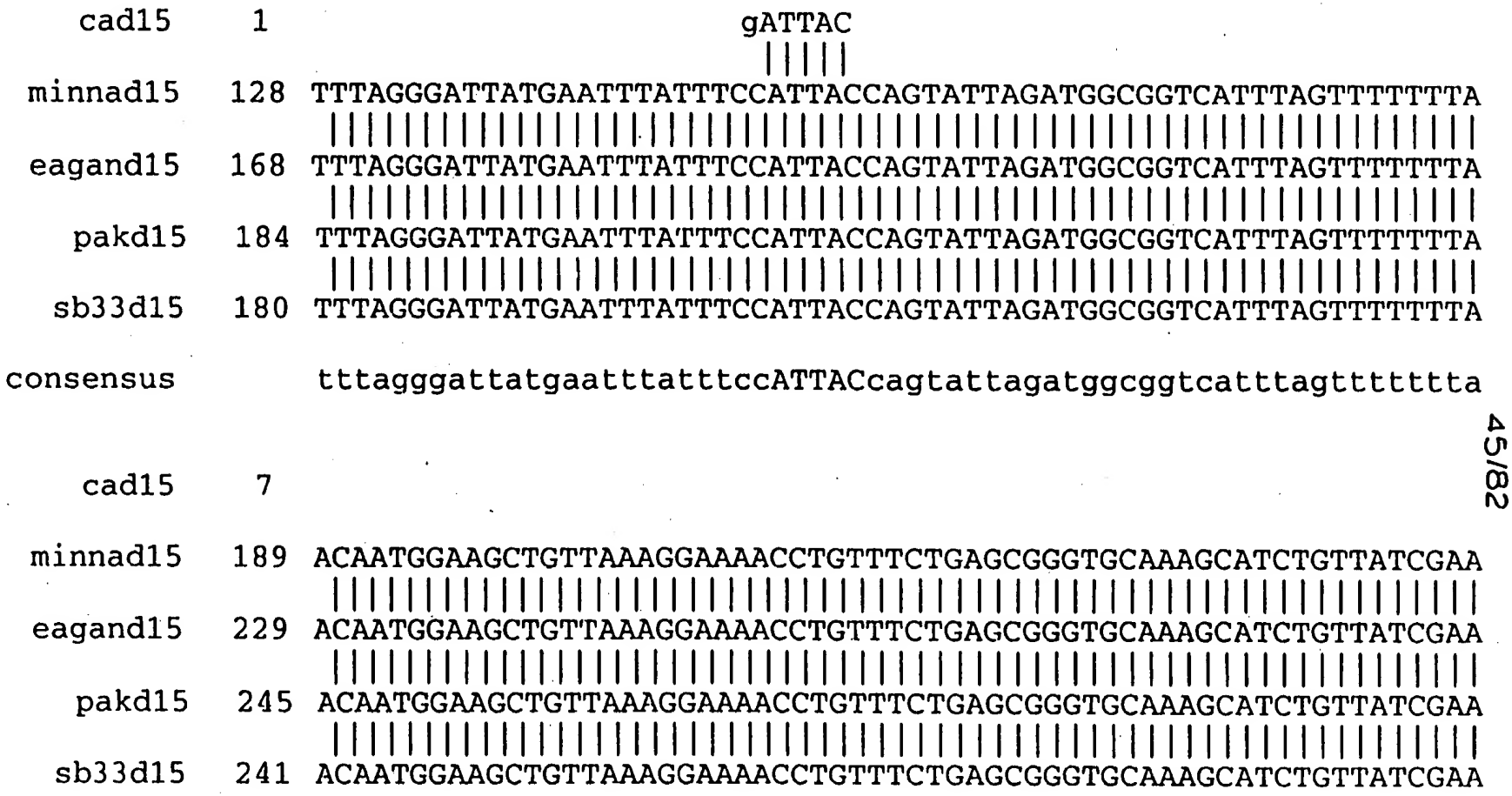


FIG.1F.(CONTINUED)

FIG.1F.(CONTINUED)

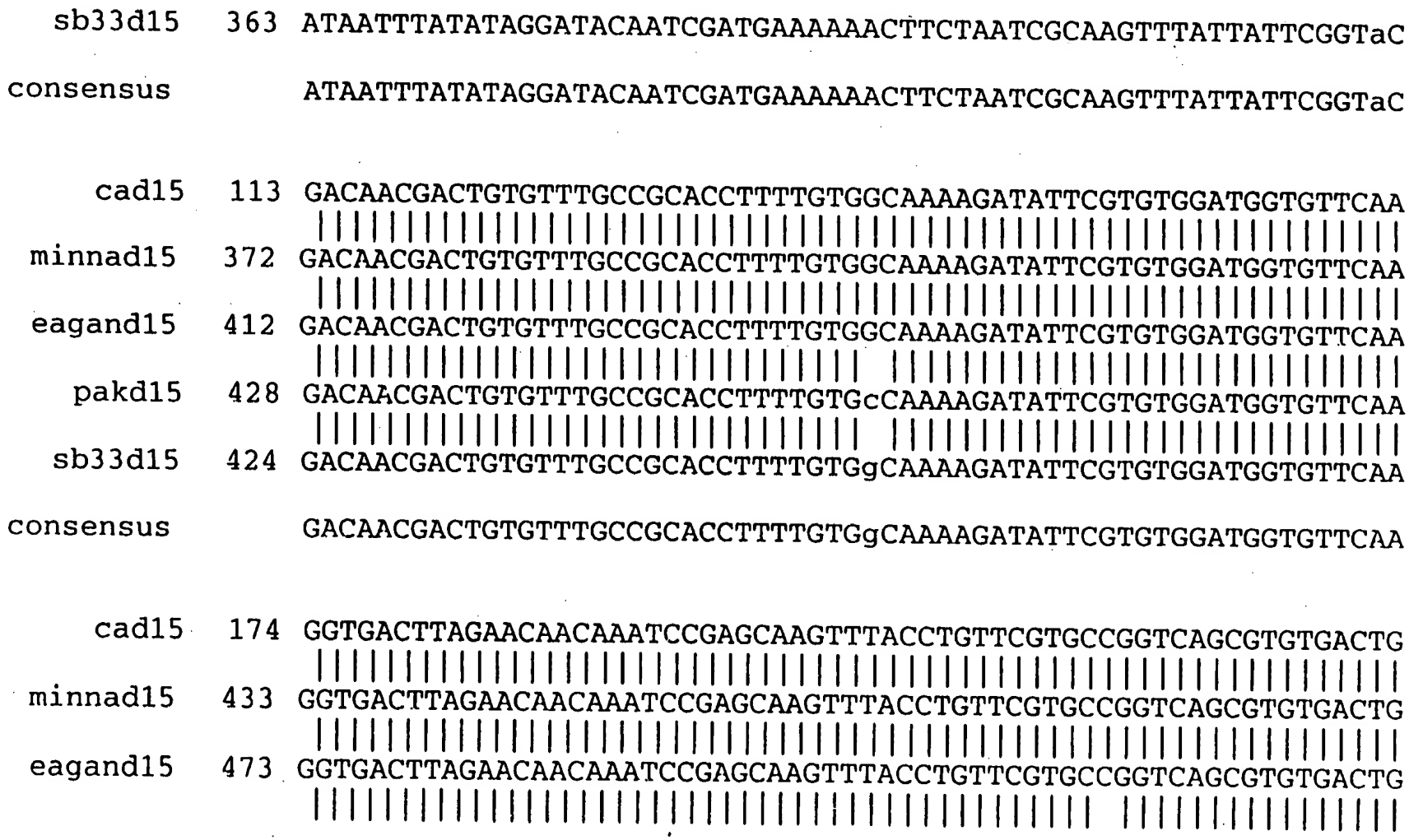


FIG.1F.(CONTINUED)

```
pakd15     489 GGTGACTTAGAACAACAAATCCGAGCAAGTTTACCTGTTCGTGCtGGTCAGCGTGTGACTG
               ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
sb33d15    485 GGTGACTTAGAACAACAAATCCGAGCAAGTTTACCTGTTCGTGCcGGTCAGCGTGTGACTG

consensus      GGTGACTTAGAACAACAAATCCGAGCAAGTTTACCTGTTCGTGCcGGTCAGCGTGTGACTG


cad15      235 ACAATGATGTGGCTAATATTGTCCGCTCTTTATTCGTAAGTGGTCGATTCGATGATGTGAA
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15   494 ACAATGATGTGGCTAATATTGTCCGCTCTTTATTCGTAAGTGGTCGATTCGATGATGTGAA
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15   534 ACAATGATGTGGCTAATATTGTCCGCTCTTTATTCGTAAGTGGTCGATTCGATGATGTGAA
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
pakd15     550 ACAATGATGTGGCTAATATTGTCCGCTCTTTATTCGTAAGTGGTCGATTCGATGATGTGAA
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
sb33d15    546 ACAATGATGTGGCTAATATTGTCCGCTCTTTATTCGTAAGTGGTCGATTCGATGATGTGAA

consensus      ACAATGATGTGGCTAATATTGTCCGCTCTTTATTCGTAAGTGGTCGATTCGATGATGTGAA


cad15      296 AGCGCATCAAGAAGGCGATGTGCTTGTTGTTAGCGTTGTGGCTAAATCGATCATTTCAGAT
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15   555 AGCGCATCAAGAAGGCGATGTGCTTGTTGTTAGCGTTGTGGCTAAATCGATCATTTCAGAT
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG.1F.(CONTINUED)

```
eagand15   595 AGCGCATCAAGAAGGCGATGTGCTTGTTGTTAGCGTTGTGGCTAAATCGATCATTTCAGAT
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
pakd15     611 AGCGCATCAAGAAGGCGATGTGCTTGTTGTTAGCGTTGTGGCTAAATCGATCATTTCAGAT
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
sb33d15    607 AGCGCATCAAGAAGGCGATGTGCTTGTTGTTAGCGTTGTGGCTAAATCGATCATTTCAGAT

consensus      AGCGCATCAAGAAGGCGATGTGCTTGTTGTTAGCGTTGTGGCTAAATCGATCATTTCAGAT


cad15      357 GTTAAAATCAAAGGTAACTCTGTTATTCCCACTGAAGCACTTAAACAAAACTTAGATGCTA
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15   616 GTTAAAATCAAAGGTAACTCTGTTATTCCCACTGAAGCACTTAAACAAAACTTAGATGCTA
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15   656 GTTAAAATCAAAGGTAACTCTGTTATTCCCACTGAAGCACTTAAACAAAACTTAGATGCTA
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
pakd15     672 GTTAAAATCAAAGGTAACTCTGTTATTCCCACTGAAGCACTTAAACAAAACTTAGATGCTA
               |||||||||||||||||||| ||||||| |||||||||| ||||||||||||||||||||
sb33d15    668 GTTAAAATCAAAGGTAACTCTaTTATTCCacCTGAAGCACTaAAACAAAACTTAGATGCTA

consensus      GTTAAAATCAAAGGTAACTCTgTTATTCCcaCTGAAGCACTtAAACAAAACTTAGATGCTA


cad15      418 ACGGGTTTAAAGTTGGCGATGTTTTAATTCGAGAAAAATTAAATGAATTTGCCAAAAGTGT
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG.1F.(CONTINUED)

```
minnad15   677  ACGGGTTTAAAGTTGGCGATGTTTTAATTCGAGAAAAATTAAATGAATTTGCCAAAAGTGT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15   717  ACGGGTTTAAAGTTGGCGATGTTTTAATTCGAGAAAAATTAAATGAATTTGCCAAAAGTGT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
pakd15     733  ACGGGTTTAAAGTTGGCGATGTTTTAATTCGAGAAAAATTAAATGAATTTGCCAAAAGTGT
                 |||||||||||||||||||| |||||||||||||||||||||||||||| |||||||
sb33d15    729  ACGGGTTTAAAGTTGGCGATaTTTTAATTCGAGAAAAATTAAATGAATTTGCCcAAAGTGT

consensus       ACGGGTTTAAAGTTGGCGATgTTTTAATTCGAGAAAAATTAAATGAATTTGCCaAAAGTGT


cad15      479  AAAAGAGCACTATGCAAGTGTAGGTCGCTATAACGCAACAGTTGAACCTATTGTCAATACG
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15   738  AAAAGAGCACTATGCAAGTGTAGGTCGCTATAACGCAACAGTTGAACCTATTGTCAATACG
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15   778  AAAAGAGCACTATGCAAGTGTAGGTCGCTATAACGCAACAGTTGAACCTATTGTCAATACG
                 |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
pakd15     794  AAAAGAGCACTATGCAAGTGTAGGTCGCTATAACGCAACCGTTGAACCTATTGTCAATACG
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
sb33d15    790  AAAAGAGCACTATGCAAGTGTAGGTCGCTATAACGCAACCGTTGAACCTATTGTCAATACG

consensus       AAAAGAGCACTATGCAAGTGTAGGTCGCTATAACGCAACaGTTGAACCTATTGTCAATACG
```

FIG.1F.(CONTINUED)

```
cad15     540 CTACCAAATAATCGCGCTGAAATTTTAATTCAAATCAATGAAGATGATAAAGCAAAATTGG
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15  799 CTACCAAATAATCGCGCTGAAATTTTAATTCAAATCAATGAAGATGATAAAGCAAAATTGG
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15  839 CTACCAAATAATCGCGCTGAAATTTTAATTCAAATCAATGAAGATGATAAAGCAAAATTGG
              || |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
pakd15    855 CTgCCAAATAATCGtGCTGAAATTTTAATTCAAATCAATGAAGATGATAAAGCAAAATTGG
              || |||||||||||| |||||||||||||||||||||||||||||||| |||||||
sb33d15   851 CTaCCAAATAATCGcGCTGAAATTTTAATTCAAATCAATGAAGATGATAAAGCcAAATTGG
consensus     CTaCCAAATAATCGcGCTGAAATTTTAATTCAAATCAATGAAGATGATAAAGCaAAATTGG

cad15     601 CATCATTAACTTTCAAGGGGAACGAATCTGTTAGTAGCAGTACATTACAAGAACAAATGGA
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15  860 CATCATTAACTTTCAAGGGGAACGAATCTGTTAGTAGCAGTACATTACAAGAACAAATGGA
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15  900 CATCATTAACTTTCAAGGGGAACGAATCTGTTAGTAGCAGTACATTACAAGAACAAATGGA
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
pakd15    916 CATCATTAACTTTCAAGGGGAACGAATCTGTTAGTAGCAGTACATTACAAGAACAAATGGA
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
sb33d15   912 CATCATTAACTTTCAAGGGGAACGAATCTGTTAGTAGCAGTACATTACAAGAACAAATGGA
consensus     CATCATTAACTTTCAAGGGGAACGAATCTGTTAGTAGCAGTACATTACAAGAACAAATGGA
```

FIG.1F.(CONTINUED)

```
cad15     662 ATTACAACCTGATTCTTGGTGGAAATTATGGGGAAATAAATTTGAAGGTGCGCAATTCGAG
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15  921 ATTACAACCTGATTCTTGGTGGAAATTATGGGGAAATAAATTTGAAGGTGCGCAATTCGAG
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15  961 ATTACAACCTGATTCTTGGTGGAAATTATGGGGAAATAAATTTGAAGGTGCGCAATTCGAG
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
pakd15    977 ATTACAACCTGATTCTTGGTGGAAATTATGGGGAAATAAATTTGAAGGTGCGCAATTCGAG
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
sb33d15   973 ATTACAACCTGATTCTTGGTGGAAATTATGGGGAAATAAATTTGAAGGTGCGCAATTCGAG

consensus     ATTACAACCTGATTCTTGGTGGAAATTATGGGGAAATAAATTTGAAGGTGCGCAATTCGAG


cad15     723 AAAGATTTGCAGTCAATTCGTGATTATTATTTAAATAATGGCTATGCCAAAGCACAAATTA
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15  982 AAAGATTTGCAGTCAATTCGTGATTATTATTTAAATAATGGCTATGCCAAAGCACAAATTA
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15 1022 AAAGATTTGCAGTCAATTCGTGATTATTATTTAAATAATGGCTATGCCAAAGCACAAATTA
              |||||| ||||| ||||||||||||||||||||||||||||||||||||||||||||| |
pakd15   1038 AAAGATcTGCAGGCAATTCGTGATTATTATTTAAATAATGGCTATGCCAAAGCACAAATCA
              |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
sb33d15  1034 AAAGATtTGCAGGCAATTCGTGATTATTATTTAAATAATGGCTATGCCAAAGCACAAATCA

consensus     AAAGATtTGCAGtCAATTCGTGATTATTATTTAAATAATGGCTATGCCAAAGCACAAATtA
```

FIG.1F.(CONTINUED)

```
     cad15   784 CTAAAACGGATGTTCAGCTAAATGATGAAAAAACAAAAGTTAATGTAACCATTGATGTAAA
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  minnad15  1043 CTAAAACGGATGTTCAGCTAAATGATGAAAAAACAAAAGTTAATGTAACCATTGATGTAAA
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  eagand15  1083 CTAAAACGGATGTTCAGCTAAATGATGAAAAAACAAAAGTTAATGTAACCATTGATGTAAA
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    pakd15  1099 CTAAAACGGATGTTCAGCTAAATGATGAAAAAACAAAAGTTAATGTAACCATTGATGTAAA
                 ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
    sb33d15 1095 CTAAAgCGGATGTTCAGCTAAATGATGAAAAAACAAAAGTTAATGTAACCATTGATGTAAA
consensus        CTAAAaCGGATGTTCAGCTAAATGATGAAAAAACAAAAGTTAATGTAACCATTGATGTAAA

     cad15   845 TGAAGGTTTACAGTATGACCTTCGTAGTGCACGCATTATAGGTAATCTGGGAGGTATGTCT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  minnad15  1104 TGAAGGTTTACAGTATGACCTTCGTAGTGCACGCATTATAGGTAATCTGGGAGGTATGTCT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  eagand15  1144 TGAAGGTTTACAGTATGACCTTCGTAGTGCACGCATTATAGGTAATCTGGGAGGTATGTCT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    pakd15  1160 TGAAGGTTTACAGTATGACCTTCGTAGTGCACGCATTATAGGTAATCTGGGAGGTATGTCT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    sb33d15 1156 TGAAGGTTTACAGTATGACCTTCGTAGTGCACGCATTATAGGTAATCTGGGAGGTATGTCT
consensus        TGAAGGTTTACAGTATGACCTTCGTAGTGCACGCATTATAGGTAATCTGGGAGGTATGTCT
```

FIG.1F.(CONTINUED)

```
cad15    906 GCCGAGCTTGAACCTTTACTTTCAGCATTACATTTAAATGATACTTTCCGCCGTAGTGATA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15 1165 GCCGAGCTTGAACCTTTACTTTCAGCATTACATTTAAATGATACTTTCCGCCGTAGTGATA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15 1205 GCCGAGCTTGAACCTTTACTTTCAGCATTACATTTAAATGATACTTTCCGCCGTAGTGATA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
pakd15   1221 GCCGAGCTTGAACCTTTACTTTCAGCATTACATTTAAATGATACTTTCCGCCGTAGTGATA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
sb33d15  1217 GCCGAGCTTGAACCTTTACTTTCAGCATTACATTTAAATGATACTTTCCGCCGTAGTGATA

consensus      GCCGAGCTTGAACCTTTACTTTCAGCATTACATTTAAATGATACTTTCCGCCGTAGTGATA


cad15    967 TTGCAGATGTAGAAAATGCAATTAAAGCAAAACTTGGAGAACGCGGTTACGGTAGCGCAAC
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15 1226 TTGCAGATGTAGAAAATGCAATTAAAGCAAAACTTGGAGAACGCGGTTACGGTAGCGCAAC
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15 1266 TTGCAGATGTAGAAAATGCAATTAAAGCAAAACTTGGAGAACGCGGTTACGGTAGCGCAAC
             |||||||||||||||||||||||||||||||||| ||||| |||||||||| | ||||
pakd15   1282 TTGCAGATGTAGAAAATGCAATTAAAGCAAAACTTGGGGAACGAGGTTACGGTAACACAAC
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
sb33d15  1278 TTGCAGATGTAGAAAATGCAATTAAAGCAAAACTTGGGGAACGAGGTTACGGTAACACAAC

consensus      TTGCAGATGTAGAAAATGCAATTAAAGCAAAACTTGGaGAACGcGGTTACGGTAgCgCAAC
```

FIG.1F.(CONTINUED)

```
     cad15  1028  GGTAAATTCAGTACCTGATTTTGATGATGCAAATAAAACATTAGCGATAACCCTTGTTGTT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   minnad15  1287  GGTAAATTCAGTACCTGATTTTGATGATGCAAATAAAACATTAGCGATAACCCTTGTTGTT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   eagand15  1327  GGTAAATTCAGTACCTGATTTTGATGATGCAAATAAAACATTAGCGATAACCCTTGTTGTT
                  ||||||||  ||||||||||||||  ||||||||||||||||||||||||||  ||||||||
     pakd15  1343  AGTAAATTCTGTACCTGATTTTGACGATGCAAATAAAACATTAGCGATAACCTTTGTTGTT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    sb33d15  1339  AGTAAATTCTGTACCTGATTTTGACGATGCAAATAAAACATTAGCGATAACCTTTGTTGTT

consensus          gGTAAATTCaGTACCTGATTTTGAtGATGCAAATAAAACATTAGCGATAACCcTTGTTGTT

     cad15  1089  GATGCTGGACGACGTTTAACTGTTCGCCAACTTCGCTTTGAAGGAAATACCGTTTCTGCTG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   minnad15  1348  GATGCTGGACGACGTTTAACTGTTCGCCAACTTCGCTTTGAAGGAAATACCGTTTCTGCTG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   eagand15  1388  GATGCTGGACGACGTTTAACTGTTCGCCAACTTCGCTTTGAAGGAAATACCGTTTCTGCTG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
     pakd15  1404  GATGCTGGACGACGTTTAACTGTTCGCCAACTTCGCTTTGAAGGAAATACCGTTTCTGCTG
                  ||||||||||||||||||||||||  ||||||||||||||||||||||||||||||||||
    sb33d15  1400  GATGCTGGACGACGTTTAACTGTTCaCCAACTTCGCTTTGAAGGAAATACCGTTTCTGCTG

consensus          GATGCTGGACGACGTTTAACTGTTCgCCAACTTCGCTTTGAAGGAAATACCGTTTCTGCTG
```

FIG.1F.(CONTINUED)

```
     cad15  1150  ATAGCACTTTACGTCAGGAAATGCGCCAACAAGAAGGAACTTGGTATAATTCACAATTAGT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   minnad15  1409  ATAGCACTTTACGTCAGGAAATGCGCCAACAAGAAGGAACTTGGTATAATTCACAATTAGT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    eagand15  1449  ATAGCACTTTACGTCAGGAAATGCGCCAACAAGAAGGAACTTGGTATAATTCACAATTAGT
                  ||||  ||||||||||||||||||||  ||||||||||||||||||||||||||||||||
      pakd15  1465  ATAGTACTTTACGTCAGGAAATGCGaCAACAAGAAGGAACTTGGTATAATTCACAATTAGT
                  ||||||||||||||||||||||||||  ||||||||||||||||||||||||||||||||
     sb33d15  1461  ATAGTACTTTACGTCAGGAAATGCGcCAACAAGAAGGAACTTGGTATAATTCACAATTAGT

consensus          ATAGcACTTTACGTCAGGAAATGCGcCAACAAGAAGGAACTTGGTATAATTCACAATTAGT

     cad15  1211  TGAGTTAGGAAAAATTCGCTTAGATCGTACAGGTTTCTTCGAAACAGTCGAAAACCGAATT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   minnad15  1470  TGAGTTAGGAAAAATTCGCTTAGATCGTACAGGTTTCTTCGAAACAGTCGAAAACCGAATT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    eagand15  1510  TGAGTTAGGAAAAATTCGCTTAGATCGTACAGGTTTCTTCGAAACAGTCGAAAACCGAATT
                  ||||||||||||||||||||||||||||||||||||||||||||  ||||||||||||||
      pakd15  1526  TGAGTTAGGAAAAATTCGCTTAGATCGTACAGGTTTCTTCGAAACAGTTGAAAACCGAATT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
     sb33d15  1522  TGAGTTAGGAAAAATTCGCTTAGATCGTACAGGTTTCTTCGAAACAGTTGAAAACCGAATT

consensus          TGAGTTAGGAAAAATTCGCTTAGATCGTACAGGTTTCTTCGAAACAGTcGAAAACCGAATT
```

FIG.1F.(CONTINUED)

```
     cad15  1272  GATCCTATCAATGGTAGTAATGATGAAGTGGATGTCGTATATAAAGTCAAAGAACGTAACA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   minnad15  1531  GATCCTATCAATGGTAGTAATGATGAAGTGGATGTCGTATATAAAGTCAAAGAACGTAACA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   eagand15  1571  GATCCTATCAATGGTAGTAATGATGAAGTGGATGTCGTATATAAAGTCAAAGAACGTAACA
                  |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
     pakd15  1587  GATCCTATCAATGGTAGCAATGATGAAGTGGATGTCGTATATAAAGTCAAAGAACGTAACA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    sb33d15  1583  GATCCTATCAATGGTAGCAATGATGAAGTGGATGTCGTATATAAAGTCAAAGAACGTAACA

consensus          GATCCTATCAATGGTAGtAATGATGAAGTGGATGTCGTATATAAAGTCAAAGAACGTAACA


     cad15  1333  CGGGTAGTATCAACTTTGGTATTGGTTACGGTACAGAGAGTGGTATTAGTTATCAAGCAAG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   minnad15  1592  CGGGTAGTATCAACTTTGGTATTGGTTACGGTACAGAGAGTGGTATTAGTTATCAAGCAAG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   eagand15  1632  CGGGTAGTATCAACTTTGGTATTGGTTACGGTACAGAGAGTGGTATTAGTTATCAAGCAAG
                  |||||||||||||||||||||||||||||||||||||||||||| |||||||| ||||
     pakd15  1648  CGGGTAGTATCAACTTTGGTATTGGTTACGGTACAGAGAGTGGTATcAGTTATCAAaCAAG
                  |||||||||||||||||||||||||||||||||||||||||||| |||||||| ||||
    sb33d15  1644  CGGGTAGTATCAACTTTGGTATTGGTTACGGTACAGAGAGTGGTATtAGTTATCAAgCAAG

consensus          CGGGTAGTATCAACTTTGGTATTGGTTACGGTACAGAGAGTGGTATtAGTTATCAAgCAAG
```

FIG.1F.(CONTINUED)

```
      cad15 1394  TGTTAAACAAGATAATTTCTTGGGAACAGGGGCGGCAGTAAGTATAGCTGGTACGAAAAAT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    minnad15 1653 TGTTAAACAAGATAATTTCTTGGGAACAGGGGCGGCAGTAAGTATAGCTGGTACGAAAAAT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    eagand15 1693 TGTTAAACAAGATAATTTCTTGGGAACAGGGGCGGCAGTAAGTATAGCTGGTACGAAAAAT
                  | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      pakd15 1709 TaTTAAACAAGATAATTTCTTGGGAACAGGGGCGGCAGTAAGTATAGCTGGTACGAAAAAT
                  | | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      sb33d15 1705 TgTcAAACAAGATAATTTCTTGGGAACAGGGGCGGCAGTAAGTATAGCTGGTACGAAAAAT
consensus         TgTtAAACAAGATAATTTCTTGGGAACAGGGGCGGCAGTAAGTATAGCTGGTACGAAAAAT

      cad15 1455  GATTATGGTACGAGTGTCAATTTGGGTTATACCGAGCCCTATTTTACTAAAGATGGTGTAA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    minnad15 1714 GATTATGGTACGAGTGTCAATTTGGGTTATACCGAGCCCTATTTTACTAAAGATGGTGTAA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    eagand15 1754 GATTATGGTACGAGTGTCAATTTGGGTTATACCGAGCCCTATTTTACTAAAGATGGTGTAA
                  |||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
      pakd15 1770 GATTATGGTACGAGTGTCAATTTGGGTTATACCGAaCCCTATTTTACTAAAGATGGTGTAA
                  |||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
      sb33d15 1766 GATTATGGTACGAGTGTCAATTTGGGTTATACCGAgCCCTATTTTACTAAAGATGGTGTAA
consensus         GATTATGGTACGAGTGTCAATTTGGGTTATACCGAgCCCTATTTTACTAAAGATGGTGTAA
```

FIG.1F.(CONTINUED)

```
     cad15  1516  GTCTTGGTGGAAATGTTTTCTTTGAAAACTACGATAACTCTAAAAGTGATACATCCTCTAA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   minnad15  1775  GTCTTGGTGGAAATGTTTTCTTTGAAAACTACGATAACTCTAAAAGTGATACATCCTCTAA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    eagand15  1815  GTCTTGGTGGAAATGTTTTCTTTGAAAACTACGATAACTCTAAAAGTGATACATCCTCTAA
                  |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
      pakd15  1831  GTCTTGGTGGAAATaTTTTCTTTGAAAACTACGATAACTCTAAAAGTGATACATCCTCTAA
                  |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
     sb33d15  1827  GTCTTGGTGGAAATgTTTTCTTTGAAAACTACGATAACTCTAAAAGTGATACATCCTCTAA

consensus          GTCTTGGTGGAAATgTTTTCTTTGAAAACTACGATAACTCTAAAAGTGATACATCCTCTAA

                                                                    59/82

     cad15  1577  CTATAAGCGTACGACTTACGGAAGTAATGTTACTTTAGGTTTCCCTGTAAATGAAAATAAC
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   minnad15  1836  CTATAAGCGTACGACTTACGGAAGTAATGTTACTTTAGGTTTCCCTGTAAATGAAAATAAC
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    eagand15  1876  CTATAAGCGTACGACTTACGGAAGTAATGTTACTTTAGGTTTCCCTGTAAATGAAAATAAC
                  |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
      pakd15  1892  CTATAAGCGTACGACTTATGGAAGTAATGTTACTTTAGGTTTCCCTGTAAATGAAAATAAC
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
     sb33d15  1888  CTATAAGCGTACGACTTATGGAAGTAATGTTACTTTAGGTTTCCCTGTAAATGAAAATAAC

consensus          CTATAAGCGTACGACTTAcGGAAGTAATGTTACTTTAGGTTTCCCTGTAAATGAAAATAAC
```

FIG.1F.(CONTINUED)

```
     cad15 1638  TCCTATTATGTAGGATTAGGTCATACCTATAATAAAATTAGTAACTTTGCTCTAGAATATA
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   minnad15 1897 TCCTATTATGTAGGATTAGGTCATACCTATAATAAAATTAGTAACTTTGCTCTAGAATATA
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    eagand15 1937 TCCTATTATGTAGGATTAGGTCATACCTATAATAAAATTAGTAACTTTGCTCTAGAATATA
                 |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
      pakd15 1953 TCCTATTATGTAGGATTAGGCCATACCTATAATAAAATTAGTAACTTTGCTCTAGAATATA
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
     sb33d15 1949 TCCTATTATGTAGGATTAGGCCATACCTATAATAAAATTAGTAACTTTGCTCTAGAATATA

consensus        TCCTATTATGTAGGATTAGGtCATACCTATAATAAAATTAGTAACTTTGCTCTAGAATATA


     cad15 1699  ACCGTAATTTATATATTCAATCAATGAAATTTAAAGGTAATGGCATTAAAACAAATGACTT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   minnad15 1958 ACCGTAATTTATATATTCAATCAATGAAATTTAAAGGTAATGGCATTAAAACAAATGACTT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    eagand15 1998 ACCGTAATTTATATATTCAATCAATGAAATTTAAAGGTAATGGCATTAAAACAAATGACTT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      pakd15 2014 ACCGTAATTTATATATTCAATCAATGAAATTTAAAGGTAATGGCATTAAAACAAATGACTT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
     sb33d15 2010 ACCGTAATTTATATATTCAATCAATGAAATTTAAAGGTAATGGCATTAAAACAAATGACTT

consensus        ACCGTAATTTATATATTCAATCAATGAAATTTAAAGGTAATGGCATTAAAACAAATGACTT
```

FIG.1F.(CONTINUED)

```
cad15 1760 TGATTTTTCTTTTGGTTGGAACTATAACAGCCTTAATAGAGGCTATTTCCCAACTAAAGGG
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15 2019 TGATTTTTCTTTTGGTTGGAACTATAACAGCCTTAATAGAGGCTATTTCCCAACTAAAGGG
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15 2059 TGATTTTTCTTTTGGTTGGAACTATAACAGCCTTAATAGAGGCTATTTCCCAACTAAAGGG
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
pakd15 2075 TGATTTTTCTTTTGGTTGGAACTATAACAGCCTTAATAGAGGCTATTTCCCAACTAAAGGG
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
sb33d15 2071 TGATTTTTCTTTTGGTTGGAACTATAACAGCCTTAATAGAGGCTATTTCCCAACTAAAGGG
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
consensus  TGATTTTTCTTTTGGTTGGAACTATAACAGCCTTAATAGAGGCTATTTCCCAACTAAAGGG
```



```
cad15 1821 GTTAAAGCAAGTCTTGGTGGACGAGTTACTATTCCAGGTTCTGATAACAAATACTACAAAC
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15 2080 GTTAAAGCAAGTCTTGGTGGACGAGTTACTATTCCAGGTTCTGATAACAAATACTACAAAC
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15 2120 GTTAAAGCAAGTCTTGGTGGACGAGTTACTATTCCAGGTTCTGATAACAAATACTACAAAC
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
pakd15 2136 GTTAAAGCAAGTCTTGGTGGACGAGTTACTATTCCAGGTTCTGATAACAAATACTACAAAC
           ||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
sb33d15 2132 GTTAAAGCAAGTCTTGGTGGACGAGTTACaATTCCAGGTTCTGATAACAAATACTACAAAC
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
consensus  GTTAAAGCAAGTCTTGGTGGACGAGTTACtATTCCAGGTTCTGATAACAAATACTACAAAC
```

FIG.1F.(CONTINUED)

```
     cad15 1882 TAAGTGCAGATGTACAGGGTTTCTACCCATTAGACAGAGATCACCTCTGGGTTGTATCTGC
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  minnad15 2141 TAAGTGCAGATGTACAGGGTTTCTACCCATTAGACAGAGATCACCTCTGGGTTGTATCTGC
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  eagand15 2181 TAAGTGCAGATGTACAGGGTTTCTACCCATTAGACAGAGATCACCTCTGGGTTGTATCTGC
                |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
    pakd15 2197 TAAGTGCAGATGTACAGGGTTTCTACCCATTAGACAGAGATCACCgCTGGGTTGTATCTGC
                |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
    sb33d15 2193 TAAGTGCAGATGTACAGGGTTTCTACCCATTAGACAGAGATCACCtCTGGGTTGTATCTGC

consensus       TAAGTGCAGATGTACAGGGTTTCTACCCATTAGACAGAGATCACCtCTGGGTTGTATCTGC

     cad15 1943 AAAAGCATCTGCAGGATATGCAAATGGTTTTGGAAACAAGCGTTTACCGTTCTATCAAACT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  minnad15 2202 AAAAGCATCTGCAGGATATGCAAATGGTTTTGGAAACAAGCGTTTACCGTTCTATCAAACT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  eagand15 2242 AAAAGCATCTGCAGGATATGCAAATGGTTTTGGAAACAAGCGTTTACCGTTCTATCAAACT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    pakd15 2258 AAAAGCATCTGCAGGATATGCAAATGGTTTTGGAAACAAGCGTTTACCGTTCTATCAAACT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    sb33d15 2254 AAAAGCATCTGCAGGATATGCAAATGGTTTTGGAAACAAGCGTTTACCGTTCTATCAAACT
```

FIG.1F.(CONTINUED)

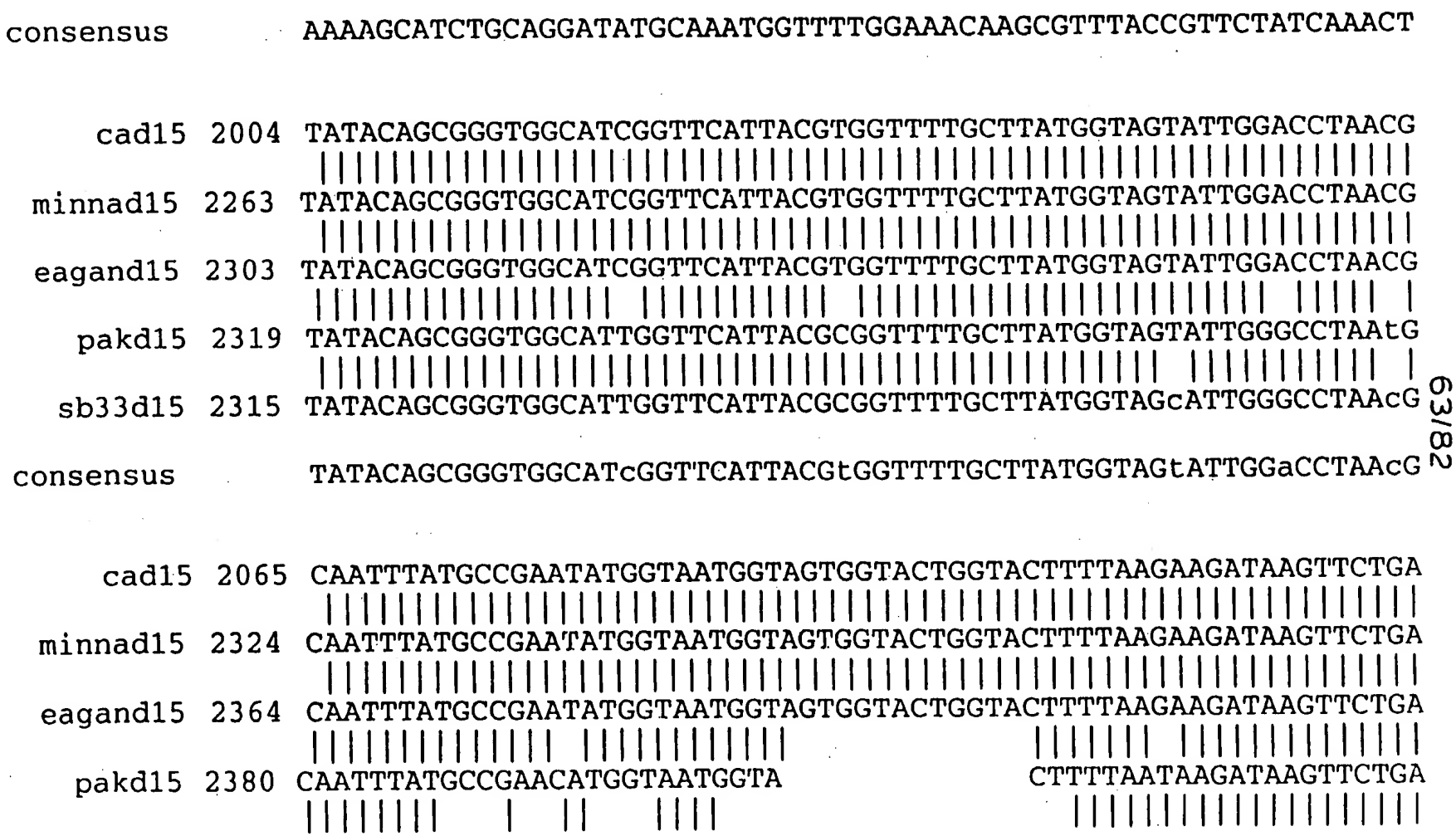


FIG.1F.(CONTINUED)

```
   sb33d15 2376 CAATTTATcaaGgtCAaaaTAAT                    aaaTTTAATAAGATAAGTTCTGA

consensus       CAATTTATgccGaatAtggTAATggtagtggtactggtactTTTAAgAAGATAAGTTCTGA


     cad15 2126 TGTGATTGGTGGTAATGCAATCGCTACAGCTAGCGCAGAGTTAATTGTGCCAACTCCATTT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  minnad15 2385 TGTGATTGGTGGTAATGCAATCGCTACAGCTAGCGCAGAGTTAATTGTGCCAACTCCATTT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  eagand15 2425 TGTGATTGGTGGTAATGCAATCGCTACAGCTAGCGCAGAGTTAATTGTGCCAACTCCATTT
                |||||||||||||||||||||| | || || || ||||| | ||||| ||||||||||||
    pakd15 2429 TGTGATTGGTGGTAATGCAATCaCaACtGCgAGtGCAGAacTtATTGTaCCAACTCCATTT
                |||||||||||||||||||||| | || || || ||||| | ||||| ||||||||||||
   sb33d15 2422 TGTGATTGGTGGTAATGCAATCgCtACaGCtAGcGCAGAgtTaATTGTgCCAACTCCATTT

consensus       TGTGATTGGTGGTAATGCAATCgCtACaGCtAGcGCAGAgtTaATTGTgCCAACTCCATTT


     cad15 2187 GTGAGCGATAAGAGCCAAAATACGGTCCGAACCTCCTTATTTGTTGATGCGGCAAGTGTTT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  minnad15 2446 GTGAGCGATAAGAGCCAAAATACGGTCCGAACCTCCTTATTTGTTGATGCGGCAAGTGTTT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  eagand15 2486 GTGAGCGATAAGAGCCAAAATACGGTCCGAACCTCCTTATTTGTTGATGCGGCAAGTGTTT
                ||||| ||||| |||||||||| |||||||||| ||||||||||||||||||||||||||
```

FIG.1F.(CONTINUED)

```
pakd15    2490  GTGAGTGATAAaAGCCAAAATACAGTCCGAACCTCCCTATTTGTTGATGCGGCAAGTGTTT
                ||||||||||| || ||||||||||||||||||||||||||||||||||||||||||||
sb33d15   2483  GTGAGTGATAAgAGtCAAAATACAGTCCGAACCTCCCTATTTGTTGATGCGGCAAGTGTTT

consensus       GTGAGcGATAAgAGcCAAAATACgGTCCGAACCTCCtTATTTGTTGATGCGGCAAGTGTTT


cad15     2248  GGAATACTAAATGGAAATCAGATAAAAATGGATTAGAGAGCGATGTATTAAAAAGATTGCC
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15  2507  GGAATACTAAATGGAAATCAGATAAAAATGGATTAGAGAGCGATGTATTAAAAAGATTGCC
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15  2547  GGAATACTAAATGGAAATCAGATAAAAATGGATTAGAGAGCGATGTATTAAAAAGATTGCC
                |||||||||||||||||||||||||||||||||||| | || || ||| || ||
pakd15    2551  GGAATACTAAATGGAAATCAGATAAAAATGGATTAGAGAGCAAgGTCTTGAAAGACTTACC
                |||||||||||||||||||||||||||||||||||| ||||||||||||||||||
sb33d15   2544  GGAATACTAAATGGAAATCAGATAAAAATGGATTAGAGAGCAAtGTCTTGAAAGACTTACC

consensus       GGAATACTAAATGGAAATCAGATAAAAATGGATTAGAGAGCgAtGTaTTaAAAagaTTgCC


cad15     2309  TGATTATGGCAAATCAAGCCGTATTCGCGCCTCTACAGGTGTCGGATTCCAATGGCAATCT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15  2568  TGATTATGGCAAATCAAGCCGTATTCGCGCCTCTACAGGTGTCGGATTCCAATGGCAATCT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

FIG.1F.(CONTINUED)

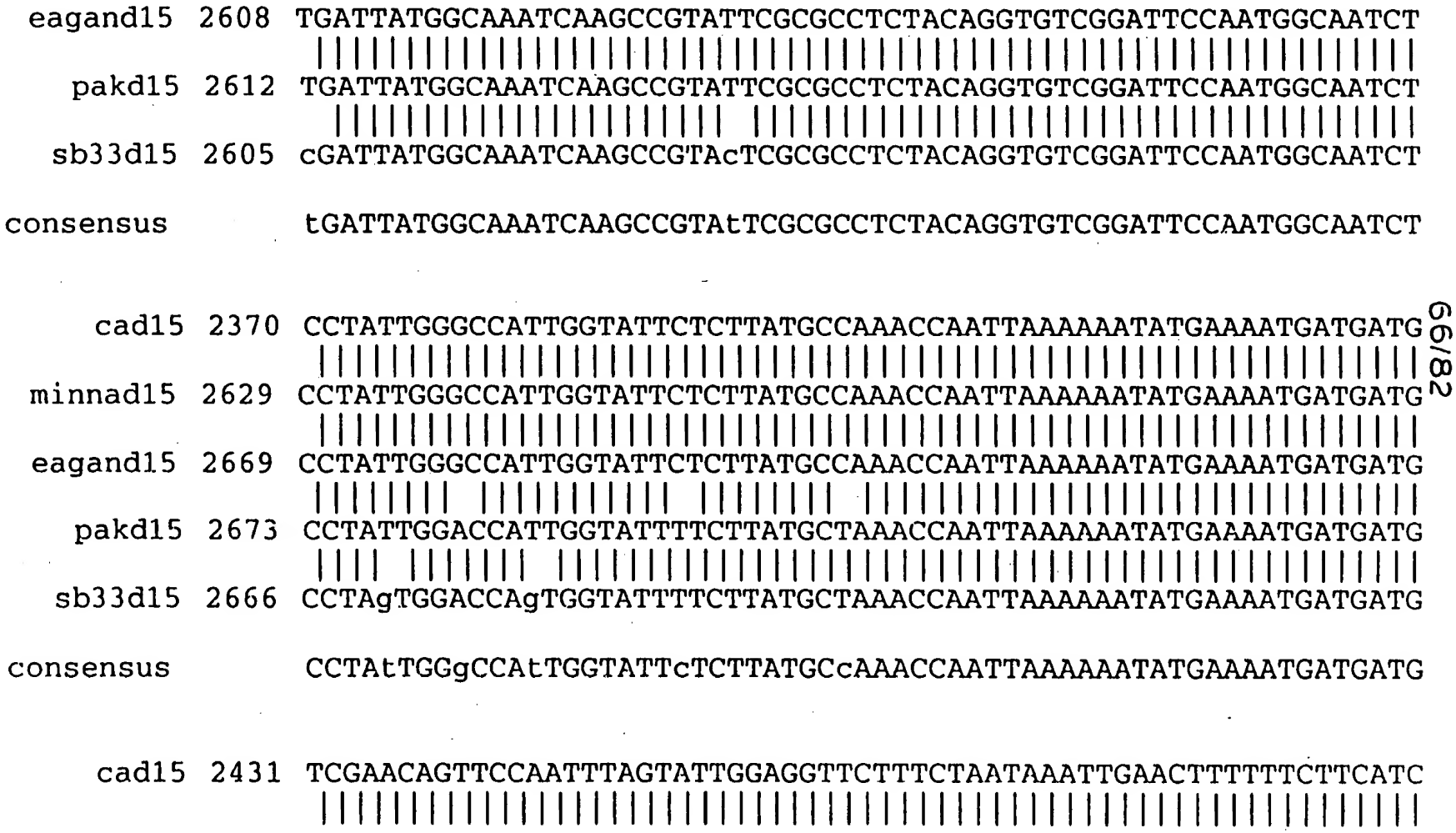


FIG.1F.(CONTINUED)

```
minnad15  2690  TCGAACAGTTCCAATTTAGTATTGGAGGTTCTTTCTAATAAATTGAACTTTTTTCTTCATC
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15  2730  TCGAACAGTTCCAATTTAGTATTGGAGGTTCTTTCTAATAAATTGAACTTTTTTCTTCATC
                |||||||||||||||||||||||| || |||||||||||||||||||||||||| ||||||
  pakd15  2734  TCGAACAGTTCCAATTTAGTATTGGGGGcTCTTTCTAATAAATTGAACTTTTTTCGTCATC
                |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
  sb33d15 2727  TCGAACAGTTCCAATTTAGTATTGGGGGtTCTTTCTAATAAATTGAACTTTTTTCGTCATC

consensus       TCGAACAGTTCCAATTTAGTATTGGaGGtTCTTTCTAATAAATTGAACTTTTTTCtTCATC


    cad15 2492  AGAACTCAAAAACAACGTTCTCTGCCTAATTTAATTGGGCAGAGAAAATATTAAACCCATC
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
minnad15  2751  AGAACTCAAAAACAACGTTCTCTGCCTAATTTAATTGGGCAGAGAAAATATTAAACCCATC
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
eagand15  2791  AGAACTCAAAAACAACGTTCTCTGCCTAATTTAATTGGGCAGAGAAAATATTAAACCCATC
                |||||||||||||| ||||||||||||||| ||||||||||||||||||||||||||||
  pakd15  2795  AGAACTCAAAAACgACGTTCTCTGCCTAATTgAATTGGGCAGAGAAAATATTAAACCCATC
                |||||||||||||| ||||||||||||||| |||||||||||||||||||| ||||||
  sb33d15 2788  AGAACTCAAAAACaACGTTCTCTGCCTAATTtAATTGGGCAGAGAAAATATTAAAaCCATC

consensus       AGAACTCAAAAACaACGTTCTCTGCCTAATTtAATTGGGCAGAGAAAATATTAAAcCCATC
```

FIG.1F.(CONTINUED)

```
     cad15  2553  ATTTAATTAAGGATATTTATCAAATGAAAAACATCGCAAAAGTAACCGCACTTGCTTTAGG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   minnad15  2812  ATTTAATTAAGGATATTTATCAAATGAAAAACATCGCAAAAGTAACCGCACTTGCTTTAGG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
   eagand15  2852  ATTTAATTAAGGATATTTATCAAATGAAAAACATCGCAAAAGTAACCGCACTTGCTTTAGG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
     pakd15  2856  ATTTAATTAAGGATATTTATCAAATGAAAAACATCGCAAAAGTAACCGCACTTGCTTTAGG
                  |||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
    sb33d15  2849  ATTTAATTAAGGATATTTATCAAATGAAAAACATCGCcAAAGTAACCGCACTTGCTTTAGG
consensus          ATTTAATTAAGGATATTTATCAAATGAAAAACATCGCaAAAGTAACCGCACTTGCTTTAGG

     cad15  2614  TATTGCACTTGCTTCAGGCTATGCTTCCGCTGAAGAAAAAATTGCTTTCATTAATGCaGGT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||
   minnad15  2873  TATTGCACTTGCTTCAGGCTATGCTTCCGCTGAAGAAAAAATTGCTTTCATTAATGCgGGT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||    |
   eagand15  2913  TATTGCACTTGCTTCAGGCTATGCTTCCGCTGAAGAAAAAATTGCTTTCATTAATGC AcT
                  | ||||||||||||||||||||||||||||||||||||||||||||||||||||| |
     pakd15  2917  TtTTGCACTTGCTTCAGGCTATGCTTCCGCTGAAGAAAAAATTGCTTTCATTAATGC AGG
                  | |||||||||||||||||||| | |||||||||||||||||||| |||||||| |||
    sb33d15  2910  TaTTGCACTTGCTTCAGGCTATGCTgCaGCTGAAGAAAAAATTGCTTTTtATTAATGC AGG
consensus          TaTTGCACTTGCTTCAGGCTATGCTtCcGCTGAAGAAAAAATTGCTTTcATTAATGC-agt
```

FIG.1F.(CONTINUED)

```
     cad15 2675 atattTTTcaAcatCacccagatcgccaagcggtagcagataaacttgatgctgaatttaa
                      |||   |    |
  minnad15 2934 TATAnTTTnCAAggCnaagg
                |||| ||| |||
  eagand15 2973 TATAtTTTTCAA
                |    ||||  |
    pakd15 2977 TTATATTTTtcAa
                |||||
   sb33d15 2970 TTATA

consensus        ttat-ttttcaaa-c-----gatcgccaagcggtagcagataaacttgatgctgaatttaa


     cad15 2736 acctgtagctgagaaattagcagcaagcaaaaaagaagttgatgataaaattgctgctgct

  minnad15 2954

  eagand15 2985

    pakd15 2990

   sb33d15 2975

consensus        acctgtagctgagaaattagcagcaagcaaaaaagaagttgatgataaaattgctgctgct
```

FIG.1F.(CONTINUED)

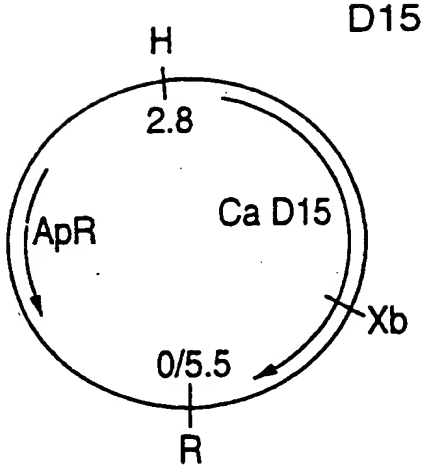
```
     cad15 2797 cgtaaaaaagtagaagcaaaagttgcggctttagaaaaagatgcacctcgcttacgtcaag
 minnad15 2954
 eagand15 2985
   pakd15 2990
  sb33d15 2975
consensus       cgtaaaaaagtagaagcaaaagttgcggctttagaaaaagatgcacctcgcttacgtcaag

     cad15 2858 ctgatattcaaaaacgccaacaggagattaataaattaggtgcggctgaagatgctgaatt
 minnad15 2954
 eagand15 2985
   pakd15 2990
  sb33d15 2975
consensus       ctgatattcaaaaacgccaacaggagattaataaattaggtgcggctgaagatgctgaatt
```

FIG.1F.(CONTINUED)

```
cad15  2919  acaaaaaattaatgcaagaacaagataaaaaa

minnad15 2954

eagand15 2985

pakd15  2990

sb33d15  2975

consensus        acaaaaaattaatgcaagaacaagataaaaaa
```

72/82

D15 CLONES



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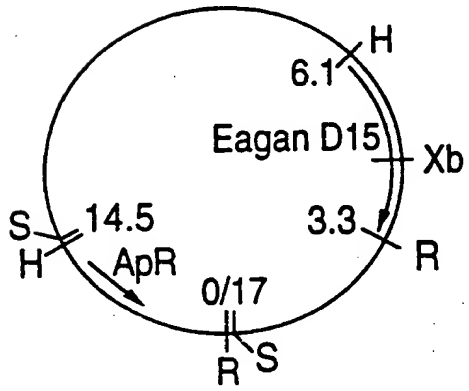

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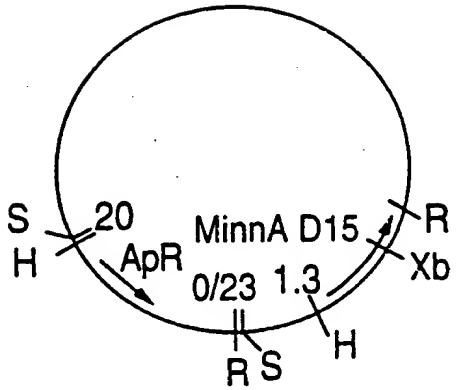

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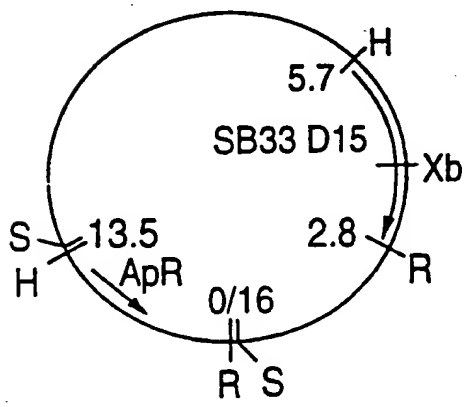

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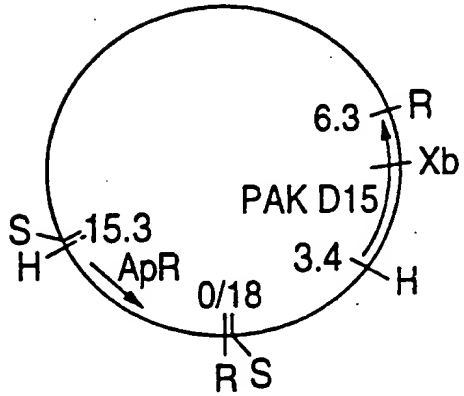


JB-1042-9-4

FIG.2.

D15 SEQUENCE COMPARISON

```
MKKLLIASLLFGITTTVFAAPFVAKDIRVDGVQGDLEQQIRASLPVRAGQRVTDNDVANIVRSLFVSGRFDDVKAHQEGDVLVVSVVAKSIISDVKIKGN  Ca
.....  Eagan
.....  MinnA
.....  SB33
.....  PAK

SVIPTEALKQNLDANGFKVGDVLIREKLNEFAKSVKEHYASVGRYNATVEPIVNILPNNRAEILIQINEDDKAKLASLTFKGNESVSSSILQEQMELQPD  Ca
.....  Eagan
.....  MinnA
.I.....I.....  SB33
.....  PAK

SWWKLWGNKFEGAQFEKDLQSIRDYYLNNGYAKAQITKTDVQLNDEKTKVNVTIDVNEGLQYDLRSARIIGNLGGMSAELEPLLSALHLNDTFRRSDIAD  Ca
.....  Eagan
.....  MinnA
.....A.....  SB33
.....A.....  PAK

VENAIKAKLGERGYGSATVNSVPDFDDANKTLAITLVVDAGRRLTVRQLRFEGNTVSADSTLRQEMRQQEGIWYNSQLVELGKIRLDRIGFFETVENRID  Ca
.....  Eagan
.....  MinnA
.....NT.....F.....H.....  SB33
.....NT.....F.....  PAK
```

FIG.3A.

```
PINGSNDEVDVVYKVKERNIGSINFGIGYGIESGISYQASVKQDNFLGIGAAVSIAGIKNDYGISVNLGYTEPYFTKDGVSLGGNVFFENYDNSKSDTSS  Ca
.....................................................................................................  Eagan
.....................................................................................................  MinnA
.....................................................................................................  SB33
.....................................T.I.....................................................I.....  PAK

NYKRTTYGSNVTLGFPVNENNSYYVGLGHTYNKISNFALEYNRNLYIQSMKFKGNGIKINDFDFSFGWNYNSLNRGYFPTKGVKASLGGRVTIPGSDNKY  Ca
.....................................................................................................  Eagan
.....................................................................................................  MinnA
.....................................................................................................  SB33
.....................................................................................................  PAK

YKLSADVQGFYPLDRDHLWVVSAKASAGYANGFGNKRLPFYQTYTAGGIGSLRGFAYGSIGPNAIYAEYGNGSGIGTFKKISSDVIGGNAIATASAELIV  Ca
.....................................................................................................  Eagan
.....................................................................................................  MinnA
.....................................................................--Q.QNNK---.N.....................  SB33
.................R.....................................................H..----...N.............T.........  PAK

PTPFVSDKSQNTVRTSLFVDAASVWNTKWKSDKNGLESDVLKRLPDYGKSSRIRASTGVGFQWQSPIGPLVFSYAKPIKKYENDDVEQFQFSIGGSF**  Ca
.....................................................................................................**  Eagan
.....................................................................................................**  MinnA
.....................................N...D.....................................................**  SB33
.....................................K...D.....................................................**  PAK
```

FIG.3B.

Construction of plasmid expressing SB33 D15

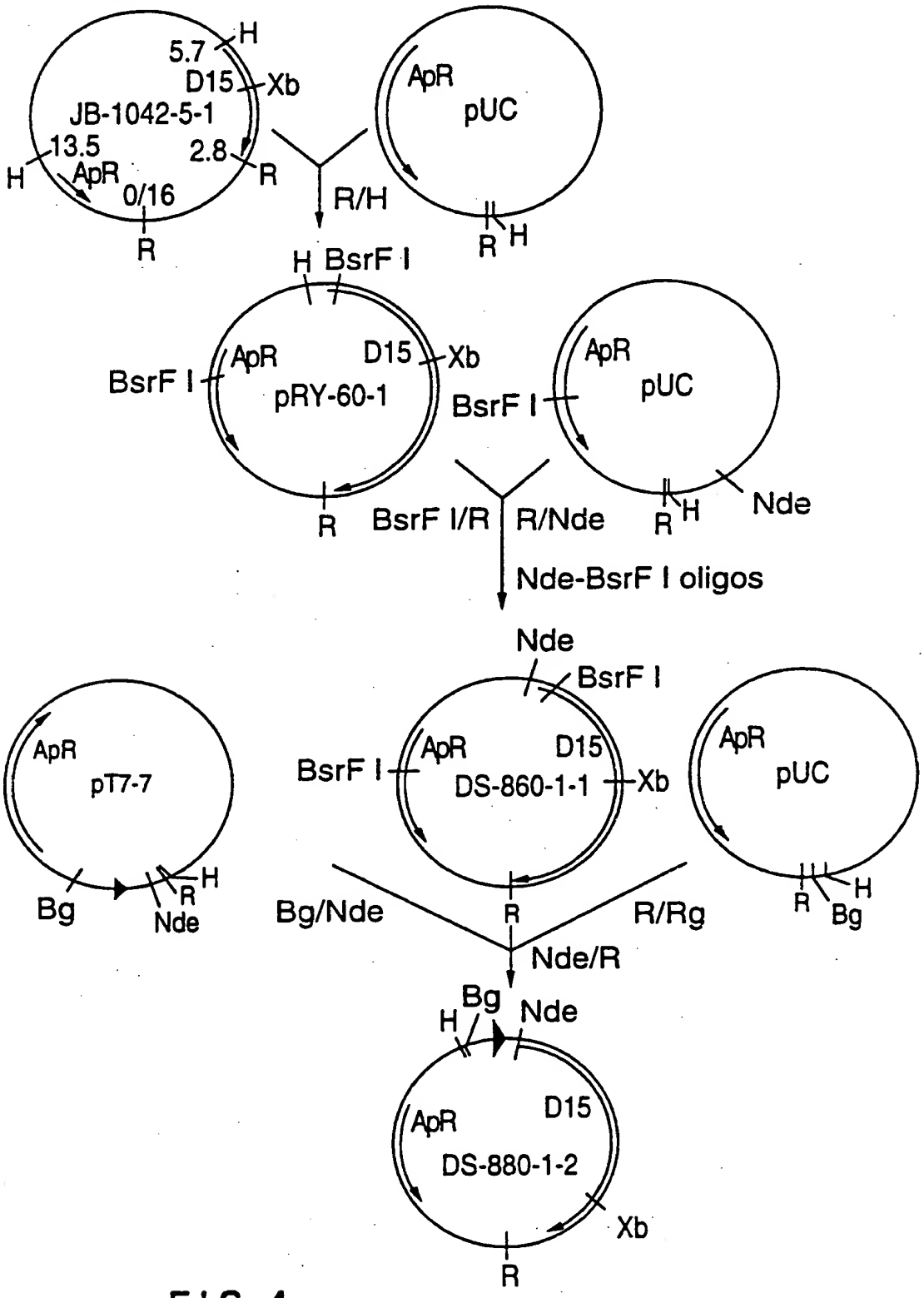
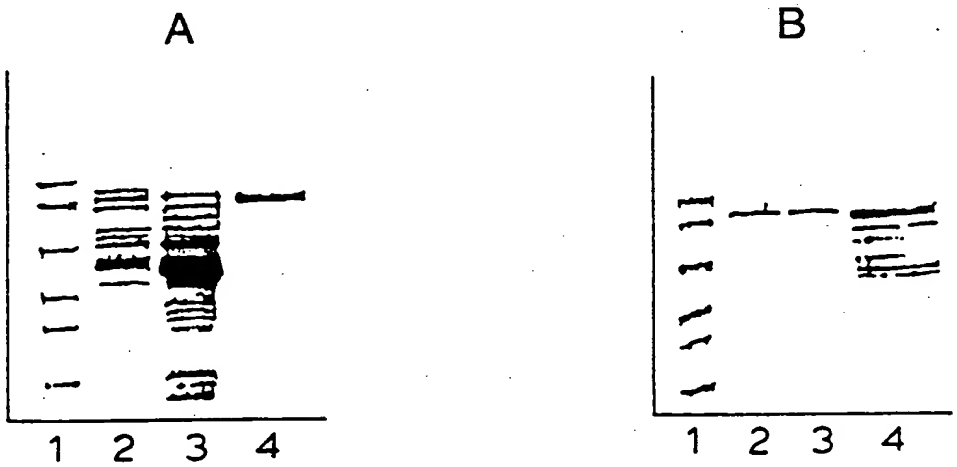


FIG.4.

PURIFICATION OF D15 FROM A NON-TYPEABLE HAEMOPHILUS INFLUENZAE STRAIN 30

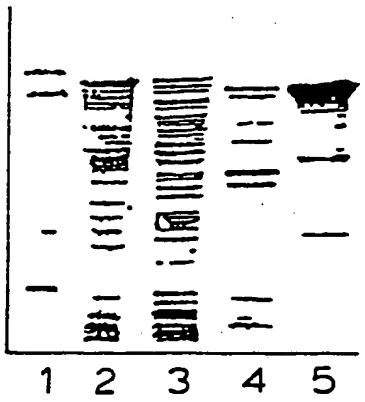


PROTEIN STAIN WESTERN BLOT

1. Low MW markers
2. Strain 30
3. Native D15 crude extract
4. D15 after anti-D15 affinity chromatography

FIG.5.

PURIFICATION OF FULL LENGTH RECOMBINANT D15



1. Protein M.W. Markers
2. Lysate of E. coli expressed rD15
3. Soluble protein in Tris-HC1 buffer extract
4. Soluble proteins in Tris/Triton X-100/ EDTA extraction buffer
5. rD15 inclusion bodies

FIG.6.

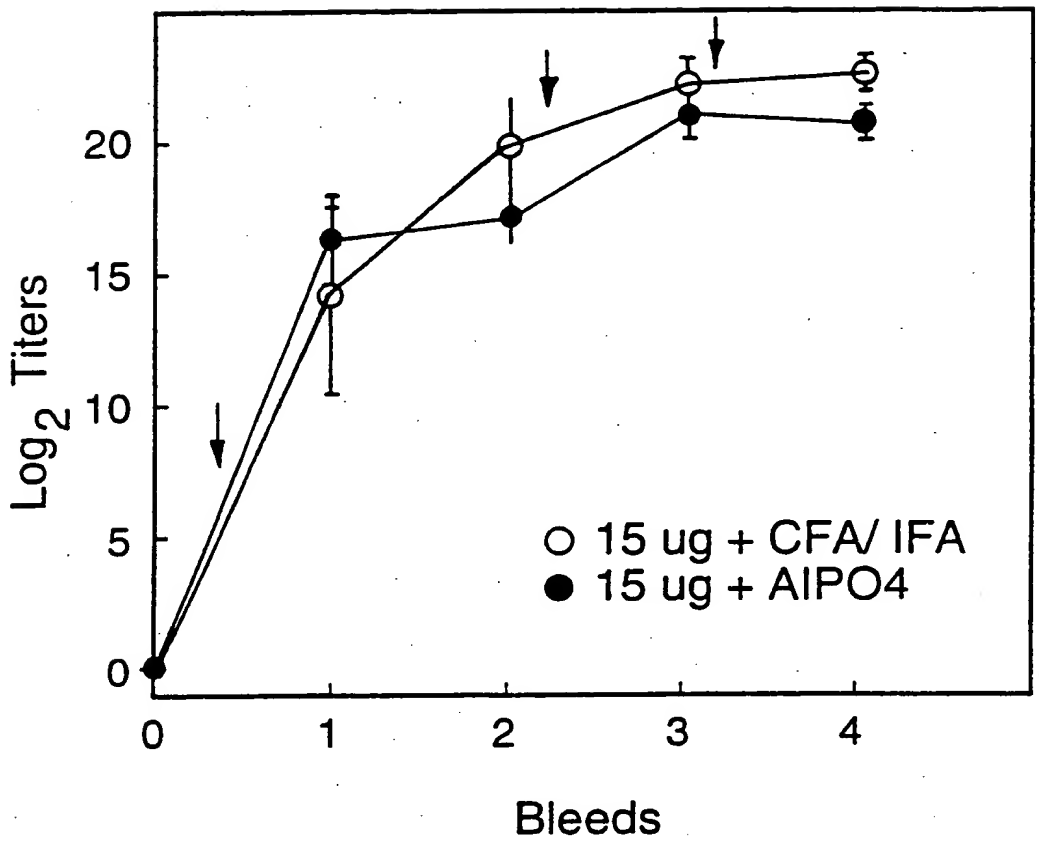


FIG.7.

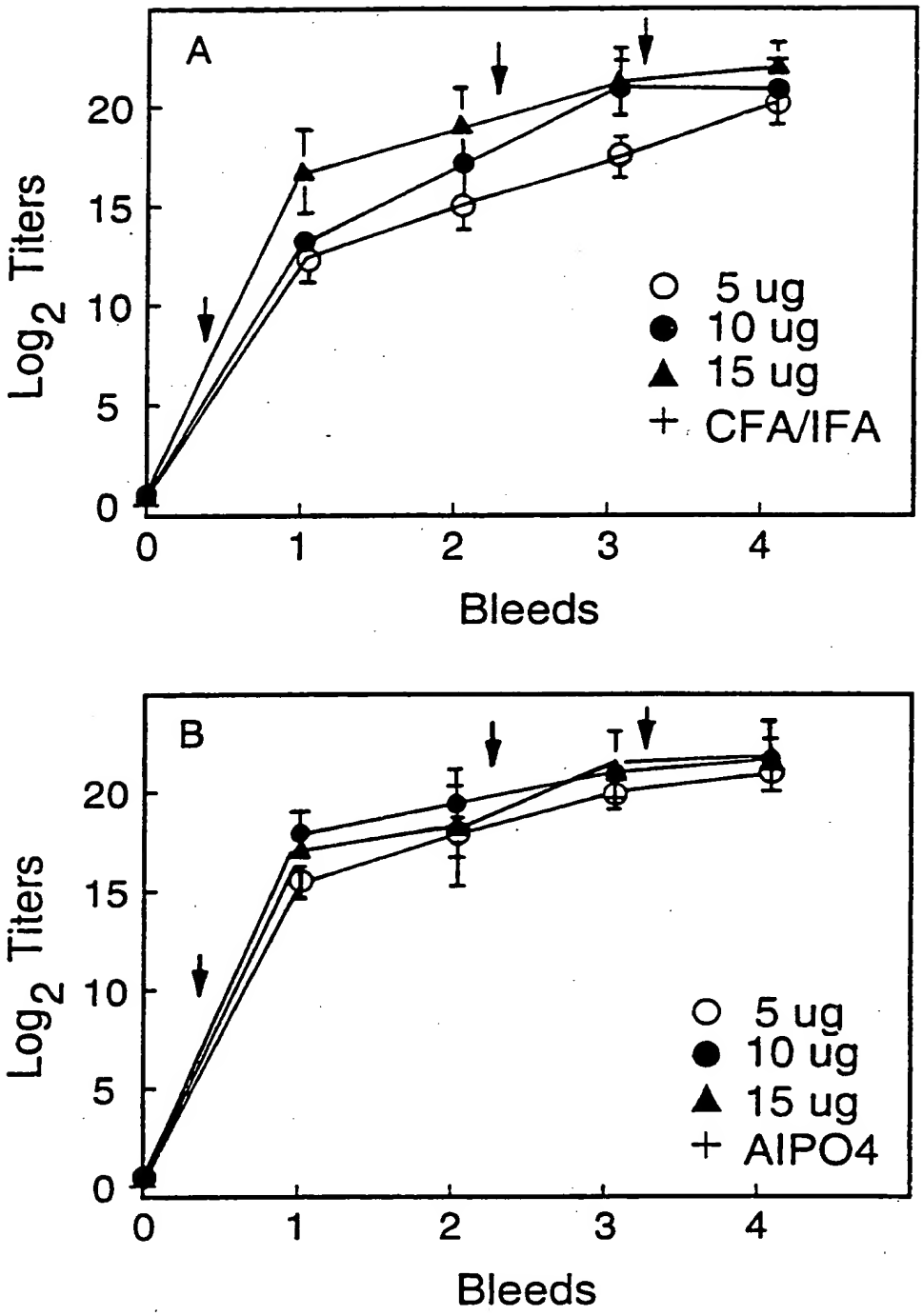
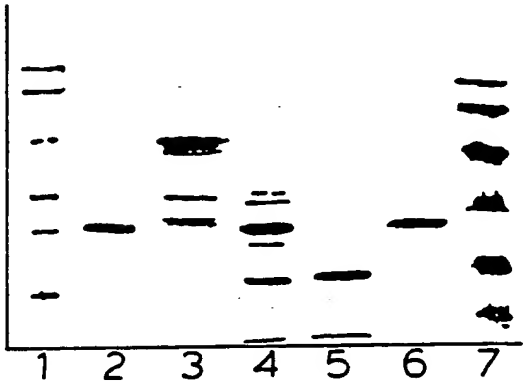

A
Log2 Titers
20
15
10
5
0
0
1
2
3
4
Bleeds
5 ug
10 ug
15 ug
CFA/IFA
B
Log2 Titers
20
15
10
5
0
0
1
2
3
4
Bleeds
5 ug
10 ug
15 ug
AlPO4

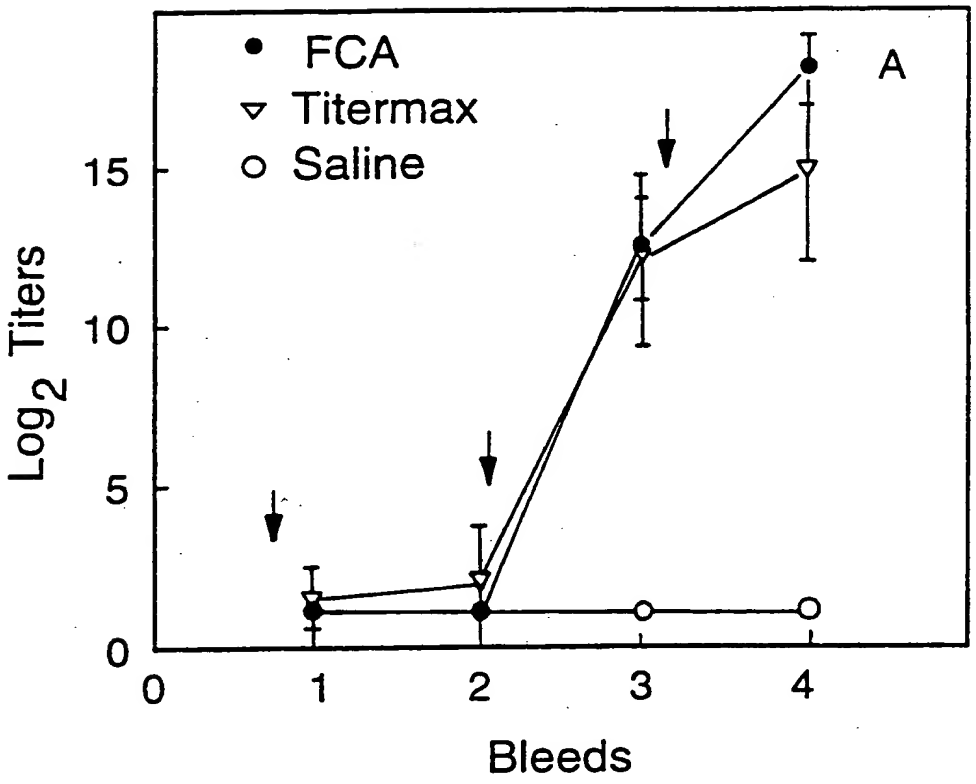
FIG.8.

PURIFICATION OF TRUNCATED D15 FROM D15-GST FUSION PROTEIN



1. Prestain low MW markers
2. GST standard
3. GST-(D15 fragment) fusion protein
4. Fusion protein cleaved by thrombin
5. rD15 fragment
6. GST
7. Low MW markers

FIG.9.


FCA
Titermax
Saline
A
Log2 Titers
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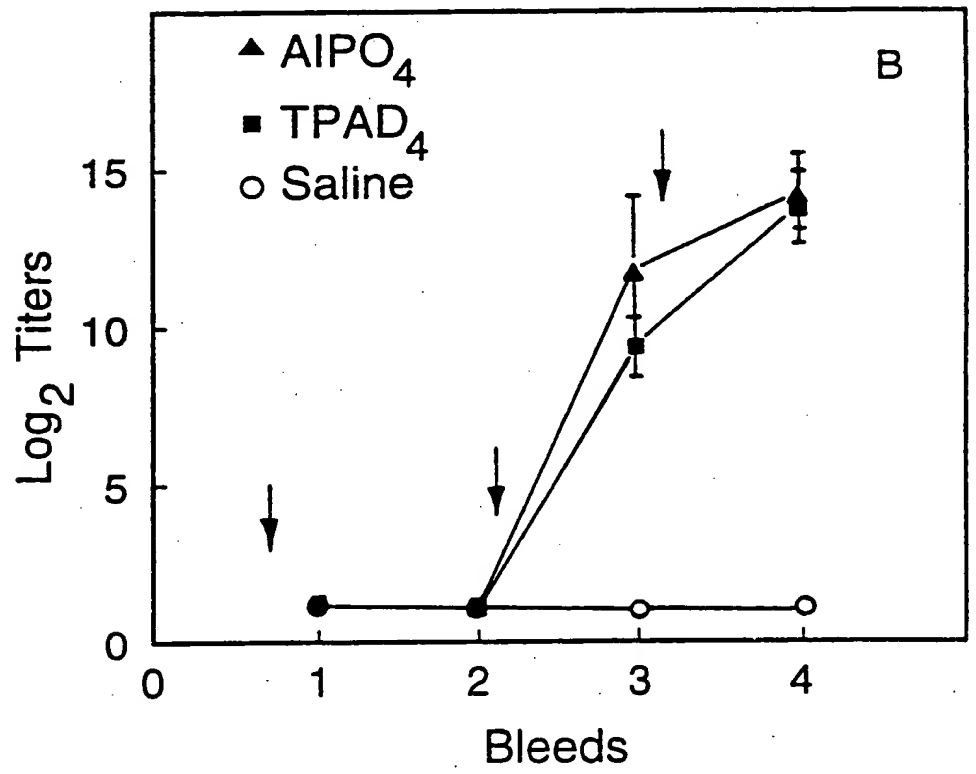
5
0
0
1
2
3
4
Bleeds


AlPO4
TPAD4
Saline
B
Log2 Titers
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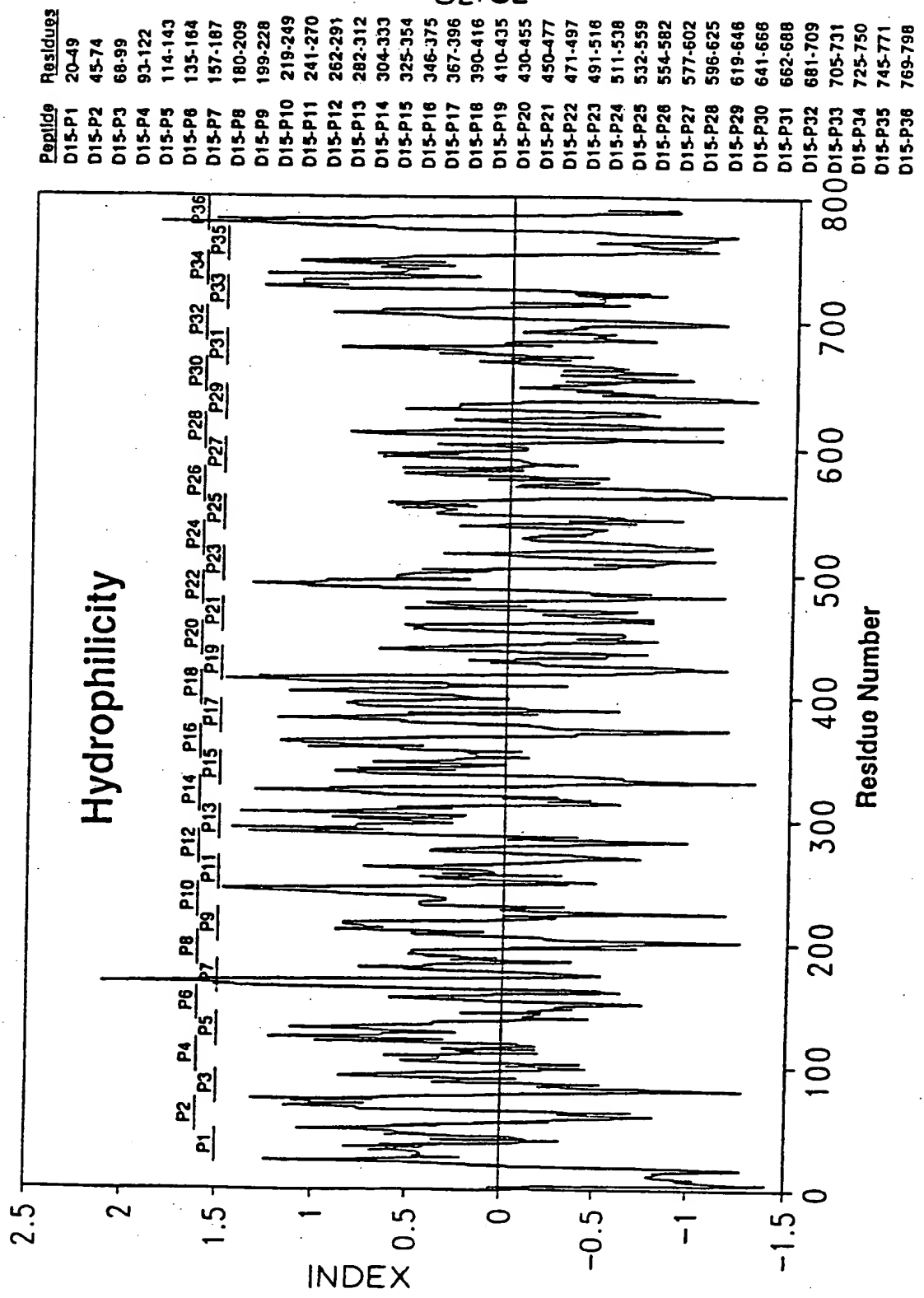
10
5
0
0
1
2
3
4
Bleeds

FIG.10.

FIGURE 11


Hydrophilicity
INDEX
Residue Number
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
0
100
200
300
400
500
600
700
800
P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11 P12 P13 P14 P15 P16 P17 P18 P19 P20 P21 P22 P23 P24 P25 P26 P27 P28 P29 P30 P31 P32 P33 P34 P35 P36
Peptide Residues
D15-P1 20-49
D15-P2 45-74
D15-P3 68-99
D15-P4 93-122
D15-P5 114-143
D15-P6 135-164
D15-P7 157-187
D15-P8 180-209
D15-P9 199-228
D15-P10 219-249
D15-P11 241-270
D15-P12 262-291
D15-P13 282-312
D15-P14 304-333
D15-P15 325-354
D15-P16 346-375
D15-P17 367-396
D15-P18 390-416
D15-P19 410-435
D15-P20 430-455
D15-P21 450-477
D15-P22 471-497
D15-P23 491-516
D15-P24 511-538
D15-P25 532-559
D15-P26 554-582
D15-P27 577-602
D15-P28 596-625
D15-P29 619-648
D15-P30 641-668
D15-P31 662-688
D15-P32 681-709
D15-P33 705-731
D15-P34 725-750
D15-P35 745-771
D15-P36 769-798

INTERNATIONAL SEARCH REPORT

ional Application No
PCT/CA 93/00501

A. CLASSIFICATION OF SUBJECT MATTER

C 12 N 15/31,C 07 K 13/00,A 61 K 39/102,
//(C 12 N 15/31; C 12 R 1/21)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K,C 07 K,C 12 N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	INFECTION AND IMMUNITY, vol. 58, no. 4, April 1990 W.R. THOMAS et al. "Expression in Escherichia coli of a High-Molecular-Weight Protective Surface Antigen Found in Nontypeable and Type b Haemophilus influenzae" pages 1909-1913, the whole document. --	1,4,6, 27
A	EP, A2, 0 378 929 (CONNAUGHT LABORATORIES LIMITED) 25 July 1990 (25.07.90), claims. --	1,4,6, 12,16, 20,22
A	WO, A1, 91/06 652	1,4,6,

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Date of the actual completion of the international search	Date of mailing of the international search report
15 February 1994	25 -03- 1994

Name and mailing address of the ISA	Authorized officer
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PCT/CA 93/00501 SAE 82214

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EP A2 378929	25-07-90	GB A0 8902178	22-03-89
		CA AA 2006587	22-06-90
		CA AA 2006587	23-06-90
		EP A3 378929	01-08-90
		JP A2 2283286	20-11-90
		GB A0 8830124	22-02-89
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		EP A1 500576	02-09-92
		GB A0 8924473	20-12-89
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US A 5013664	07-05-91	keine - none - rien	